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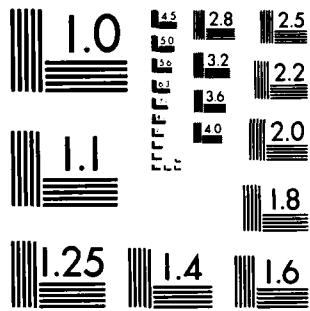
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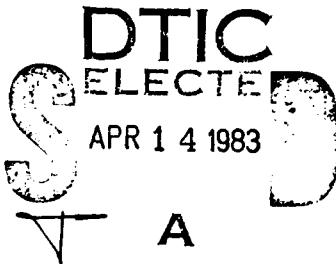
STUDIES OF PHLEBOTOMIN₁ SAND FLIES

ANNUAL REPORT

By

D. G. YOUNG

31 August 1980



Supported by

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DEPARTMENT OF ENTOMOLOGY & NEMATOLOGY
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)	A preliminary key to the phlebotomine sand flies of Kenya was prepared as an aid to identification. The need for fresh material became readily apparent. Further progress was made on illustrating important features of New World species, necessary for inclusion in a forthcoming handbook of the American sand flies. New geographic records and specimens for the reference collection were given to the Principal Investigator by colleagues working in Guatemala, Mexico and Brazil. Several descriptive papers were submitted for	

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publication and another, a review of a new species group (Microps Group), was nearly finished. Attempts to rear and maintain three U.S. phlebotomine species were successful. A key problem of high larval mortality faced by many investigators was overcome by the development of a single diet discussed in a short note submitted for publication.



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ABSTRACT

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7. Key Words: Sand fly

Lutzomyia

Phlebotominae

Phlebotomus

Leishmaniasis

Note: Copies of this report are filed with the Defense Document Center, Cameron Station, Alexandria, Virginia 33214, and may be obtained by qualified investigators working under government contract.

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PROGRESS REPORT

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Introduction

The importance of phlebotomine sand flies as vectors of leishmaniasis and arboviruses in many areas of the world is considerable. The W.H.O. scientific working group on leishmaniasis (1977 report) stated that ". . . taxonomic studies of these vectors are of fundamental importance due to the difficulty in identifying them." Recommendations of this group included continued maintenance of phlebotomine reference collections and support for research programs dealing with systematics of these insects. The group stressed the "need for new practical field keys" in some areas.

Objectives

The objectives under this contract are similar to those outlined by the W.H.O. working group. In addition to studying the American species (the projected date of completion of a handbook on these is June, 1982), we began to study the phlebotomines of Africa, beginning with Kenya, and the Middle East. Objectives include:

1. Preparing keys, illustrations, and other aids to identification both by geographic areas and by taxonomic groups.
2. Arriving at a more satisfactory classification of the subfamily phlebotominae.
3. Building a reference collection on a worldwide basis.
4. Maintaining one or more species in laboratory colonies to provide

immature stages and adults necessary for the evaluation of non-morphological taxonomic techniques.

Results

Slide mounted specimens of Kenyan phlebotomines (27 species) in our collection enabled the Principal Investigator and Capt. Raymond Beach to compile a preliminary identification key to the species known to occur there (Appendix I). The specimens on hand were poorly mounted over 20 years ago in temporary medium; therefore, it was not possible to critically evaluate all of the taxonomic characters believed to be important. A forthcoming trip to that country during June-July, 1981, working in cooperation with investigators at the International Centre of Insect Physiology and Ecology, Nairobi, as an invited scientist and WRAIR should be fruitful in terms of obtaining adequate samples of various species. One of the unsolved taxonomic problems of direct importance to the epidemiology of visceral leishmaniasis in Kenya is the apparent impossibility of separating the 3 *Synphlebotomus* spp. females by conventional means. One of these, *Phlebotomus martini* is the suspected vector but *P. celiae* may also be involved.

Work continued on the handbook of the New World species. The emphasis is being placed on identification; thus, many illustrations will be included--a time consuming but necessary aspect of the project. Most of the time spent on this handbook during the past year involved making drawings.

Colleagues sent specimens to the PI from Mexico, Guatemala, Brazil and the U.S.A. for determination. About 1000 flies were slide mounted and identified; those from Mexico and Guatemala, both little-collected

areas, were especially interesting. Dr. Charles Porter, CDC medical entomologist in Guatemala, collected 9 species previously unknown in the Republic. These are *Brumptomyia hamata*, *B. galindoi*, *Lutzomyia odax*, *L. ovallesi*, *L. carpenteri*, *L. shannoni*, *L. texana*, *L. trinidadensis* and *L. panamensis*. The latter species is an incriminated vector of cutaneous leishmaniasis in Panama.

Mexican material, collected at Miacatlán (Morelos) by Dr. Marco Camino and sent to the PI, is valuable because this locality is close to the type locality of 3 poorly known taxa--*L. durani*, *L. hardisoni* and *L. dodgei*. These species and at least 7 others, including the common *L. longipalpis*, were added to our reference collection. Information on these species will be included in the handbook.

Descriptions of two Amazonian species were sent to press (Appendices II and III). One of these forms, *L. olmeca nociva*--a man biter, belongs in the *flaviscutellata* complex which includes all the known vectors of *Leishmania mexicana* from Mexico to Brazil. The review of the *Lutzomyia davisi* complex should be published by April, 1981. A paper on a new group, the *Microps* group with 5 South American Species, was nearly completed.

Lutzomyia shannoni, a man-biting species in parts of its widespread geographic range (U.S.A.-Argentina), was collected for the first time in South Carolina and at Ft. Bragg, North Carolina, by LTC Ron Intermill in 1980. The identifications were made by the PI and CPT Peter Perkins, Ph.D. graduate student working with the PI [the fact that *shannoni* occurs at Ft. Bragg where *Leishmania*-infected soldiers apparently live and train indicates that transmission from vector to man or other mammal could occur]. The recent identification of *Leishmania infantum*, an Old World visceral

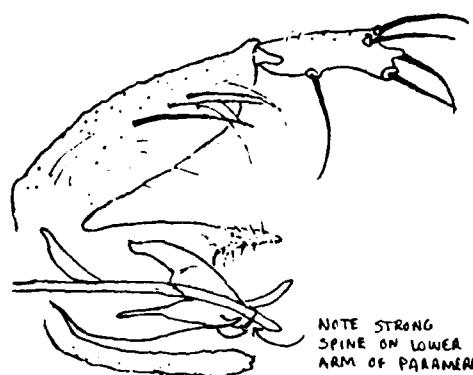
Leishmania, in Oklahoma in dogs (LTC Larry Hendricks, personal comm.), indicates that the successful establishment of an imported infection in the U.S.A. is possible. *L. shannoni* and two other U.S. species were successfully reared and colonized in our laboratory, mainly by graduate students working with the PI. Better methods of rearing resulted in a scientific note which was submitted to the Journal of Medical Entomology in 1980 (Appendix IV). The importance of rearing these insects in relation to taxonomy is clearly evident when morphologically inseparable, sibling species are considered. These methods for rearing immatures and handling adults of the *Synphlebotomus* complex in Kenya will be tried. If successful, then males and females in this complex can be properly associated, and identified specimens can be studied by various techniques, including cuticular hydrocarbon analysis.

Specimens of *L. anthophora* were sent to the Department of Arboviral Entomology, Ft. Detrick, for subcolonization and subsequent vector competence studies. The importance of maintaining phlebotomine colonies, especially vectors, is considerable--not only for taxonomic studies but also for experimental work not covered under this contract.

Appendix I

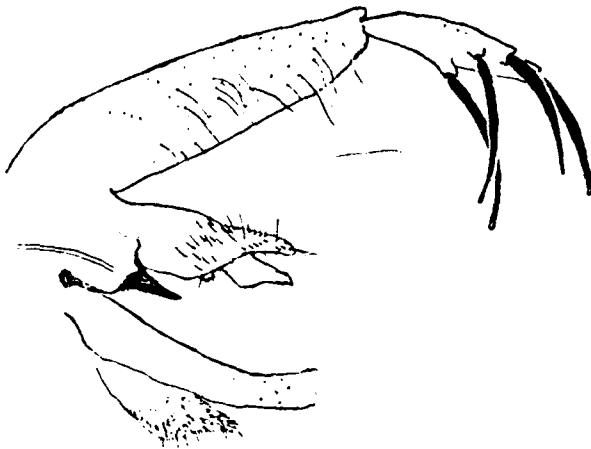
PRELIMINARY KEY TO THE PHLEBOTOMINAE OF KENYA

Males



P. HEISCHI

NOTE STRONG
SPINE ON LOWER
ARM OF PARAMER

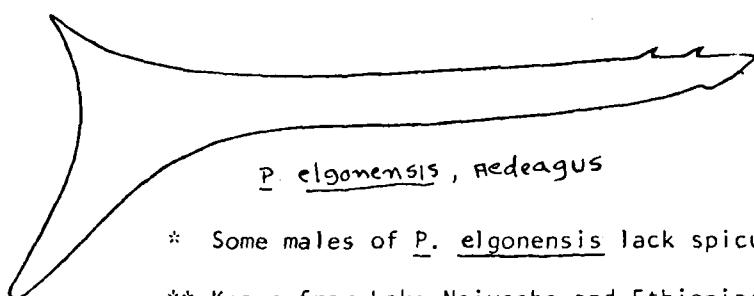


P. rodhaini

Genital filaments shorter than 3x length of pump. Coxite tuft of persistent hairs inserted on long arm. (Subgenus Synphlebotomus) . . . 8

* SEE COUPLET 6 OF FEMALE KEY FOR FIGURE OF PLEURAL SETAE.

4. Aedeagus with 1 or more subterminal spicules* 5
Aedeagus without such spicules. 6



P. elgonensis, Aedeagus



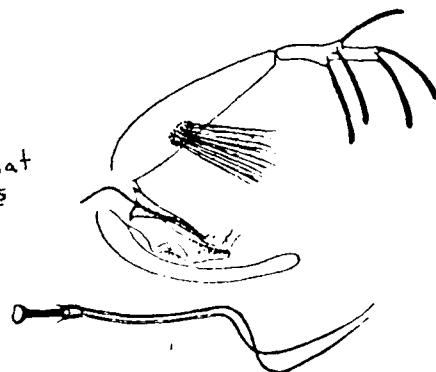
P. aculeatus

* Some males of P. elgonensis lack spicules.

** Known from Lake Naivasha and Ethiopia; elgonensis known only from caves on western slope of Mt. Elgon (2100 M to 2500 M).

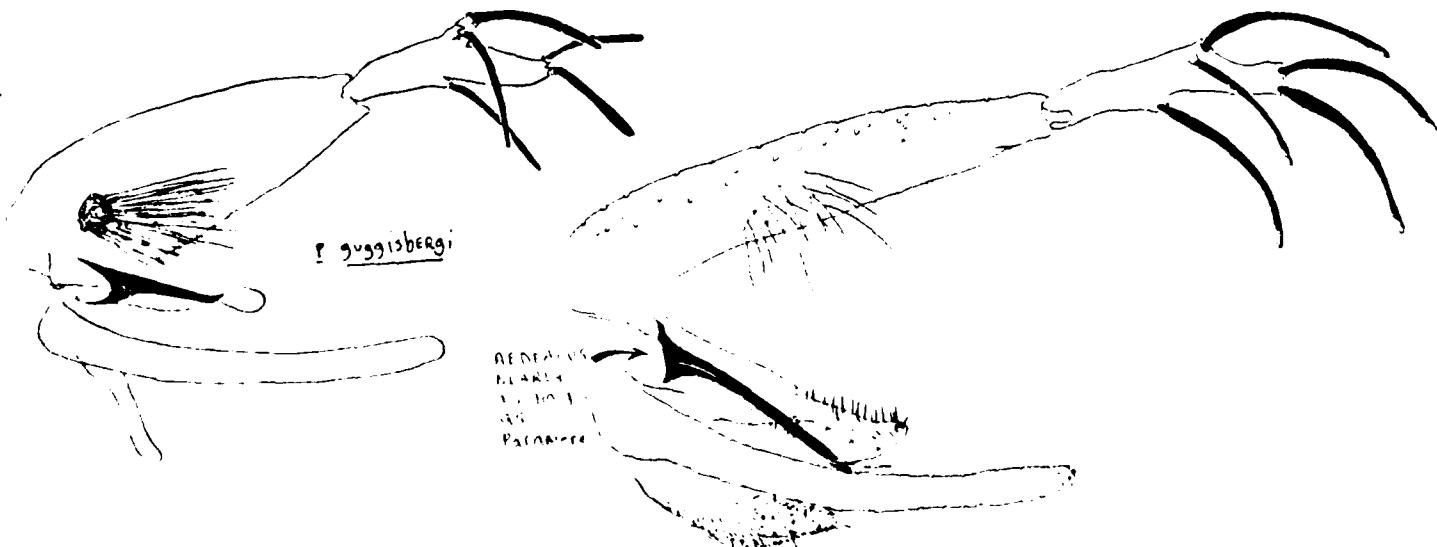
5. 2/III-VII, 1/VIII-XV. Wing length 2.9-3.0 P. aculeatus**
2/III-XV. Wing length 3.2 - 3.44 P. elgonensis

GENITALIA OF
aculeatus; that
of elgonensis
LOOKS VERY
MUCH LIKE
IT.



6. Coxite setae inserted on raspberry-like patch P. guggisbergi

Coxite setae more scattered, not inserted on a slightly raised patch. . . 7



7. Aedeagus nearly as long as parameres, its tip straight or nearly so. . .

..... P. langeroni orientalis*

Aedeagus shorter than parameres, its tip turned upwards . . . P. pedifer**

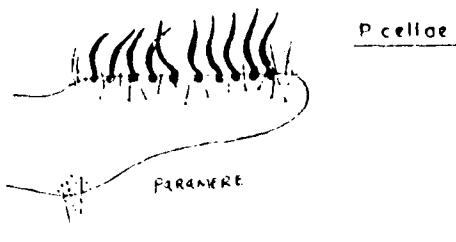


AEDEAGUS
OF P PEDIFER-
UP TURNED AT
TIP

8. Coxite setae of 7 or so spatulate hairs and some thin ones. Parameres

with dorsal sickle-shaped setae. P. celiae

Coxite and paramere setae simple 9



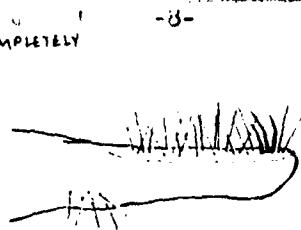
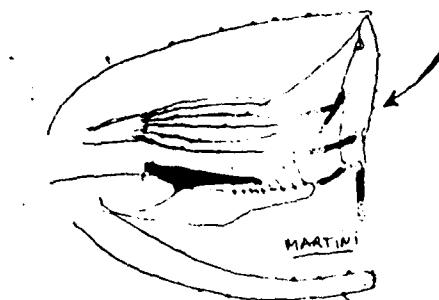
9. Coxite tuft of 6 flat hairs and about 12 thin ones. P. martini

Coxite tuft of 10 flat hairs and fewer than 12 thin ones

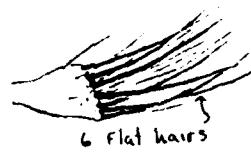
..... P. vansomerenae

* Vector of Kala-azar in Sudan. Also called P. orientalis by various authors.

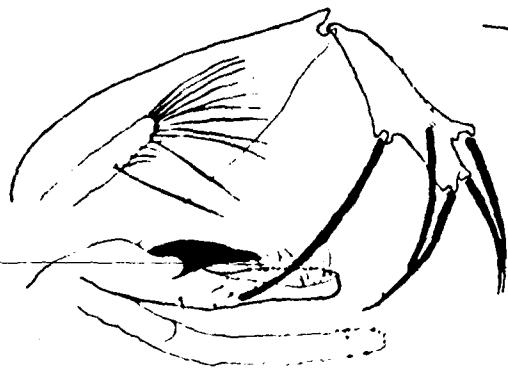
** Vector of cutaneous Leishmaniasis in Kenya.



P. martini



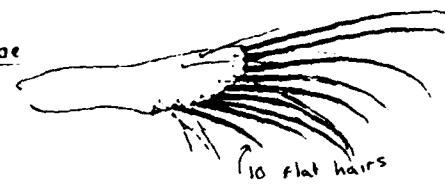
6 Flat hairs



VANSOMERENAE



P. vansomerenae



10 flat hairs

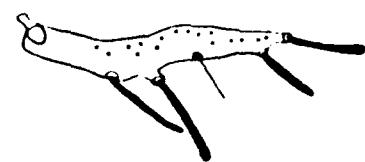
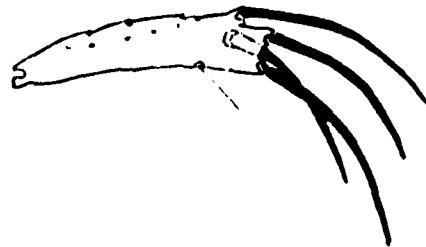
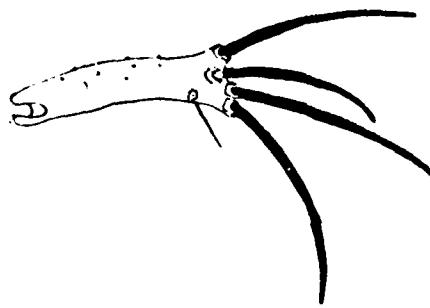
10. Paramere forked P. rodhaini (FIGURE ON PAGE 1)
 Paramere simple 11

11. Coxite with persistent setae inserted on a long arm . . . P. saevus
 Coxite with persistent setae usually absent but, if present, not
 inserted on an arm or tubercle. (Genus Sergentomyia, in part) 12



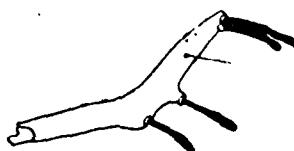
P. saevus

12. Style with 2 terminal spines and 2 median (more or less) major spines . . . 13
 Style with all major spines well beyond middle of segment 17

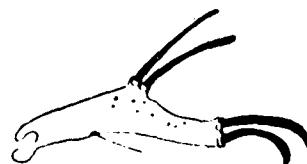


13. Style with 2 median spines well separated 14

Style with 2 median spines adjacent, close together 15



S. kirki

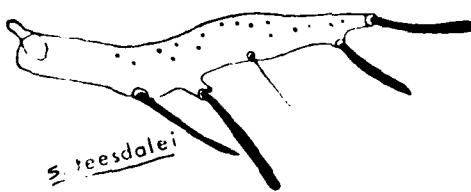


S. ingrami

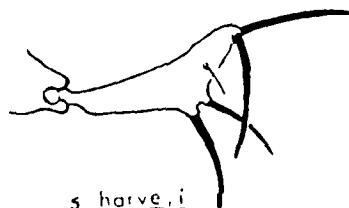
14. Style with 2 terminal spines at end S. kirki

Style with 1 terminal spine at end, the other subterminal

. S. teesdalei



S. teesdalei



S. harveyi

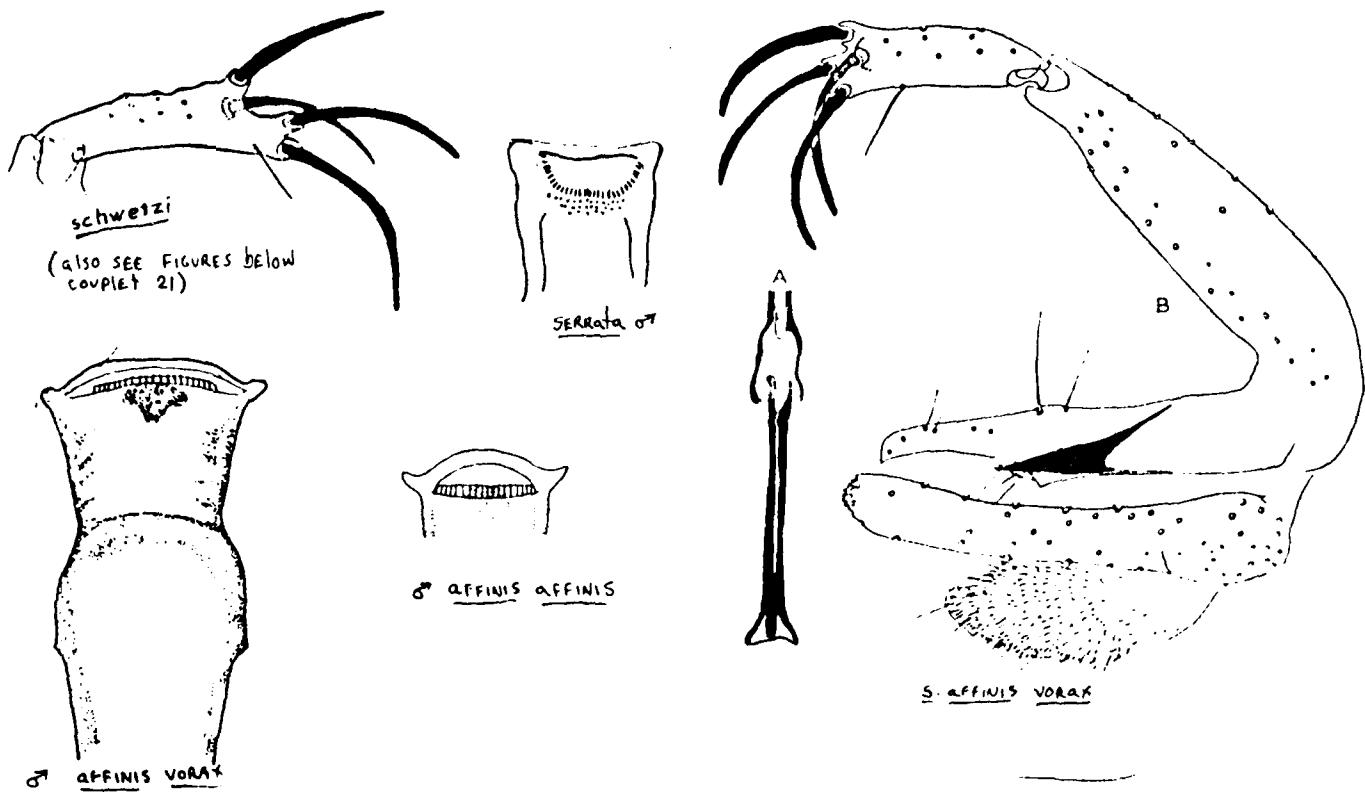
15. Style with small seta between the median and terminal groups of major spines S. harveyi

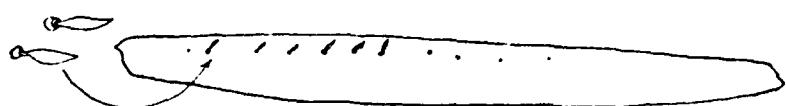
Style with small seta proximal to, or on same level as, median pair

of major spines 16

16. Cibarium with ca. 35 straight pointed teeth arranged in an arc;
vertical teeth punctiform in 2-3 rows S. serrata
Cibarium with ca. 30 very short teeth; few or no punctiform vertical
teeth S. ingrami

17. Style with 2 terminal spines and 2 others at distal 3/4
. S. schwetzi (typical form)
Style with all spines terminal or subterminal, i.e. close together. . . 18

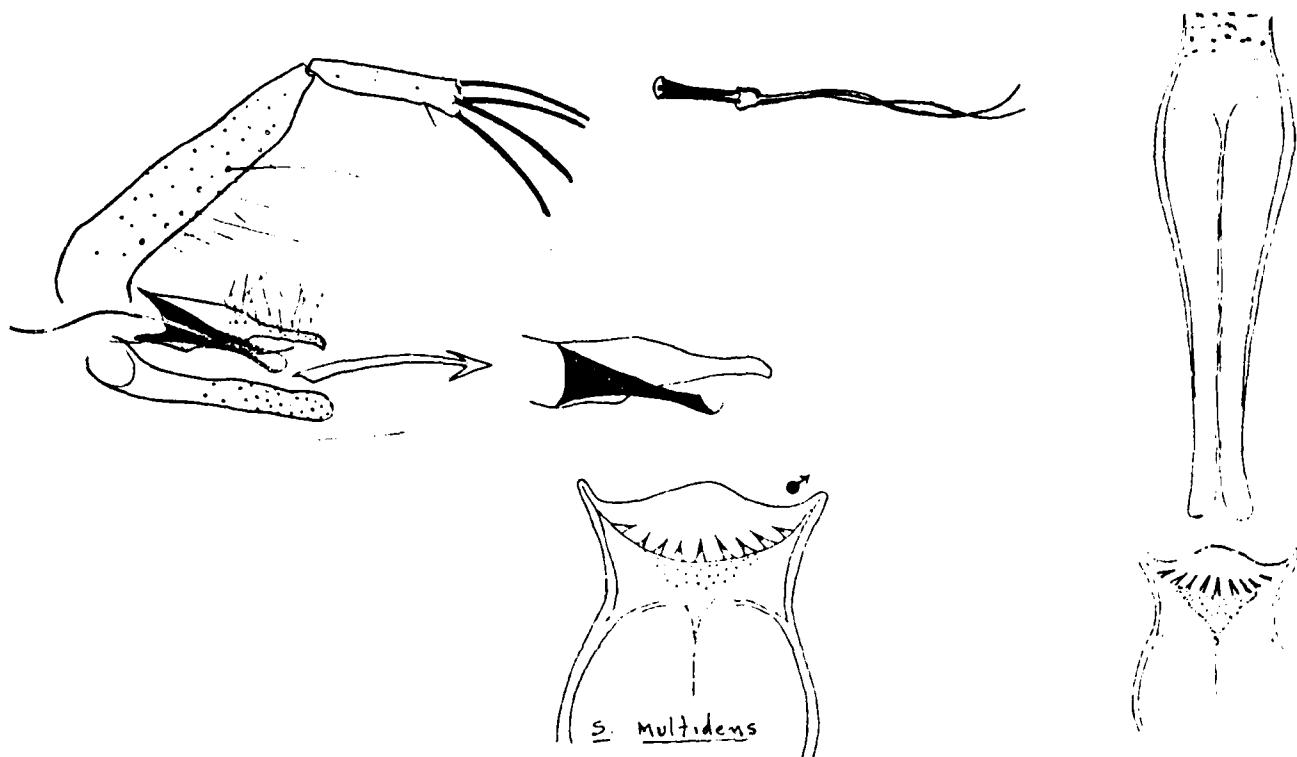




S. AFFINIS AFFinis and *S. AFFINIS VORAS* occur in areas that are close together.

19. Aedeagus with pale tip, rounded, dialated S. multidens

Aedeagus with pointed, dark tip 20



20. Aedeagus finger-shaped 21

Aedeagus conical 24



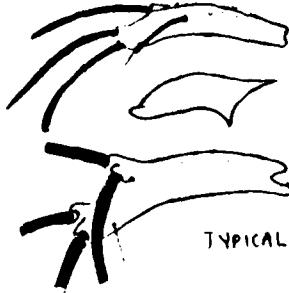
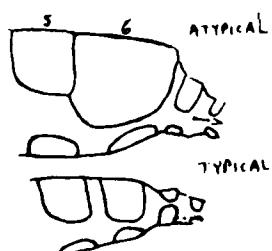
FINGER-SHAPED

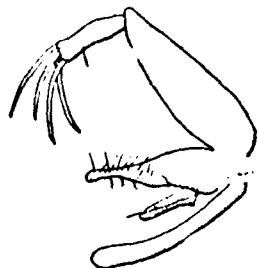


SHORT CONICAL

21. 6th abdominal tergite longer than 5th (atypical form) S. schwetzi*

tergite
6 abdominal about same size as 5th 22



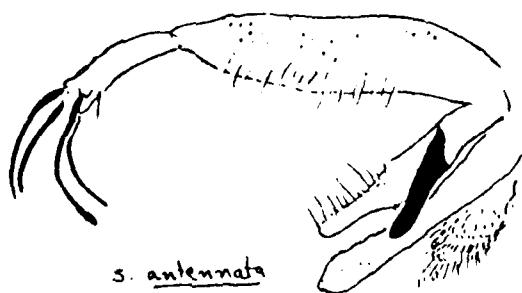


S. Yusaf

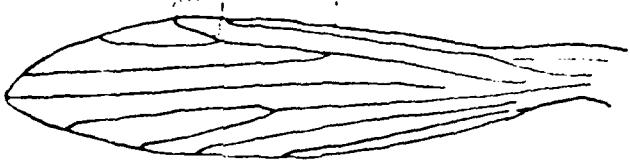


S. Yusaf

THERE MAY BE SEVERAL FORMS OF
ANTENNATA IN KENYA. TO KEY THEM
USE ABONNENC (1972). S. CINCTA IS ONE
OF THESE FORMS.

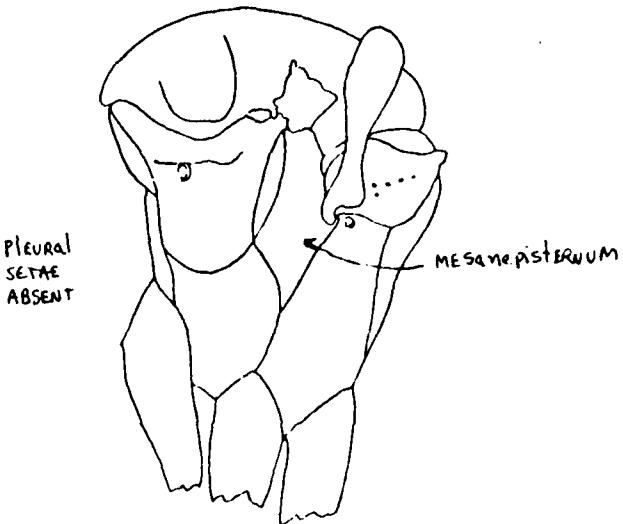


3. antennata

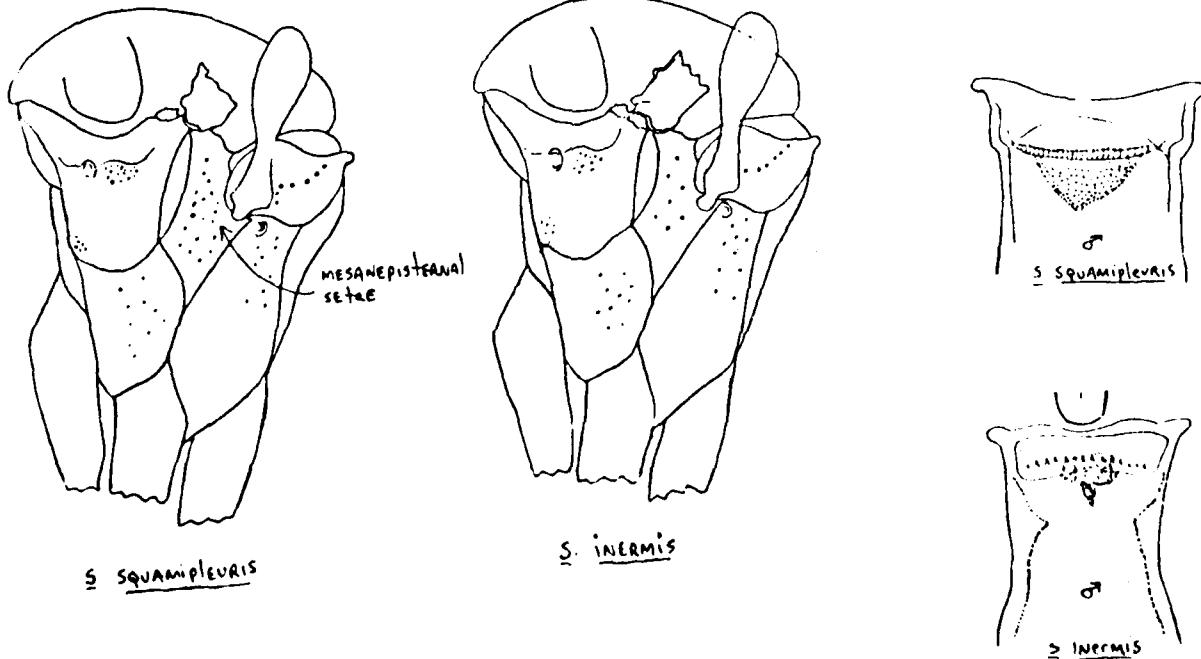


Santennata



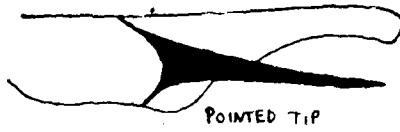
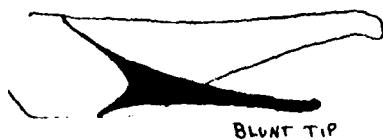


* NOTE. SOME MALES OF
SERGENTONYIA AFRICANA
magna have pleural
SETAE, but they are NEVER
ON THE MESANEPISTERNUM.
SEE FIGURE BELOW COUPLET
20 IN FEMALE KEY
WHICH SHOWS THE POSITION
OF THESE SETAE.

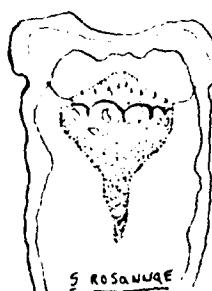
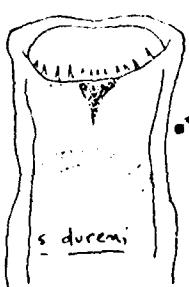
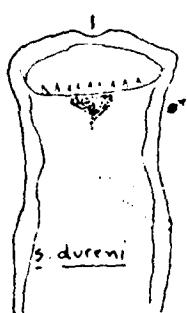


* * COMMONLY ATTRACTED TO LIGHT THERE IS AN ATYPICAL FORM WITH ENLARGED 6TH ABDOMINAL TERGITE.

26. Aedeagus with blunt tip 27
Aedeagus with pointed tip 30

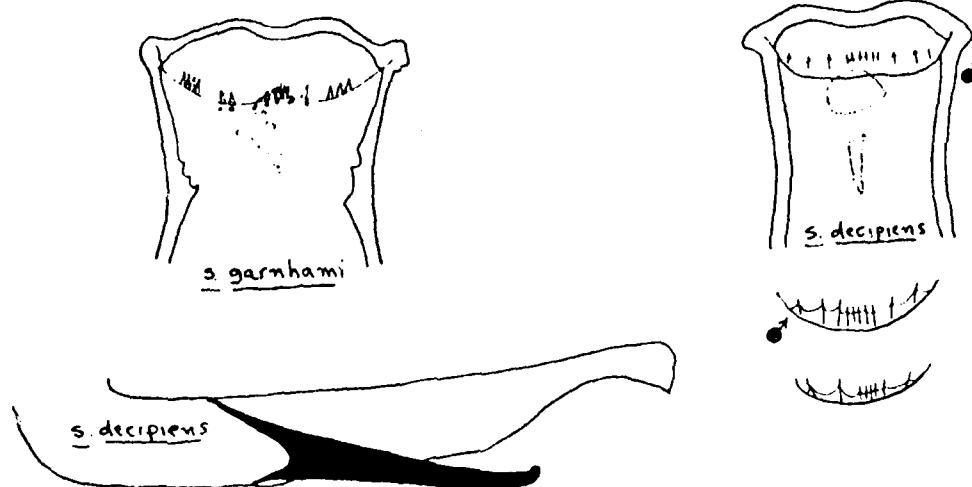


27. Antennal segment III (0.18 - 0.25 mm long), equal to or shorter than labrum 28
Antennal segment III (0.30 - 0.50 mm long), longer than labrum ... 29
29. Pigment patch well developed with tooth-like thickenings
S. rosannae
Pigment patch invisible or nearly so, no such
thickenings S. dureni



30. Antennal segment III very long (0.40-0.50 long). Cibarium with 15 short needle-like teeth spaced unevenly along a weakly concave posterior arc S. garnhami*

Antennal segment III shorter (0.30-0.36 mm long). Cibarium with 4-12 unequal teeth; 3 median ones short and narrow with long and stout lateral teeth S. decipiens



30. Palpal formula 1-2-4-3-5. Antennal segment III shorter or equal to labrum 31

Palpal formula 1-2-3-4-5. Antennal segment III longer than labrum 32

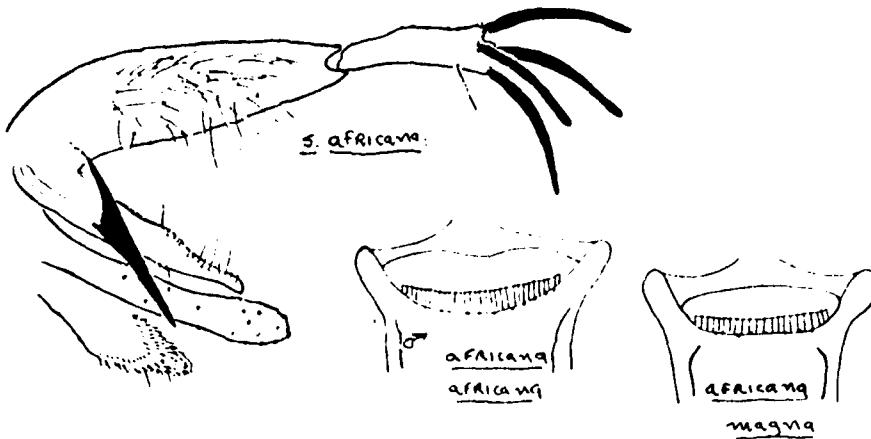
31. Cibarium with 6-7 groups of well-developed denticles. Abdominal tergite 6 considerably larger than 5 S. clydei

Cibarium with 10-12 distinct teeth and 2-3 rows of anterior punctiform denticles. Tergites 5 and 6 subequal in size S. adleri

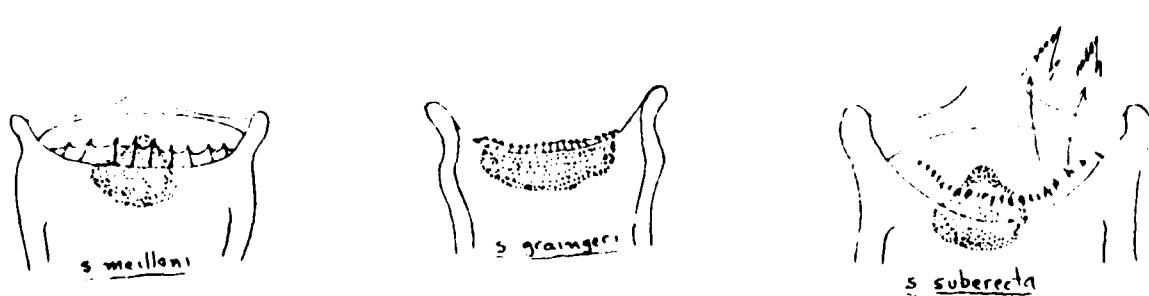


* CLYDEI sometimes bites man, as does garnhami

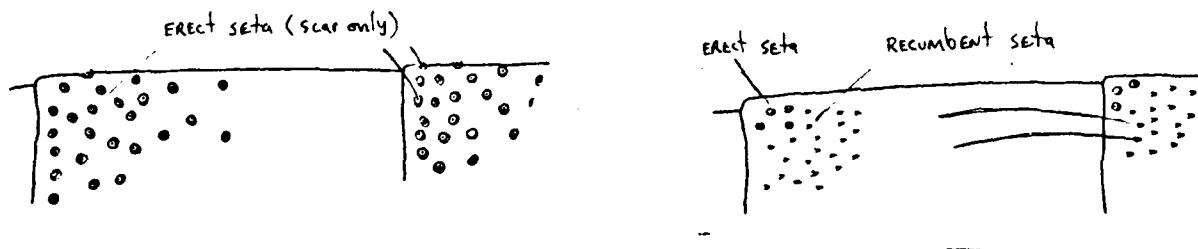
32. Antennal segment III < 0.28 mm. No pigment plate. Coxite with numerous median and distal setae. Pleural setae present or not .. 33
Antennal segment III > 0.28 mm. Pigment plate present. Coxite with fewer setae. Pleural setae absent 34
33. Pleural setae usually present (see figure below; couplet 20 in female key). Cibarium with 14-23 teeth S. africana magna
Pleural setae absent. Cibarium with 26-36 teeth S. africana africana

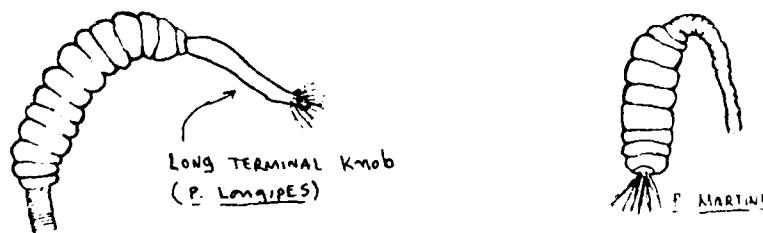


34. Cibarium with a row of 9 needle-like teeth S. meilloni
Cibarium with a row of 17-25 teeth 35
35. Cibarium with a row of 17-20 very short teeth in a palisade; pigment patch short and broad S. graingeri
Cibarium with 18-25 fringe-like teeth; pigment patch shaped like a "top" S. suberecta



Females*



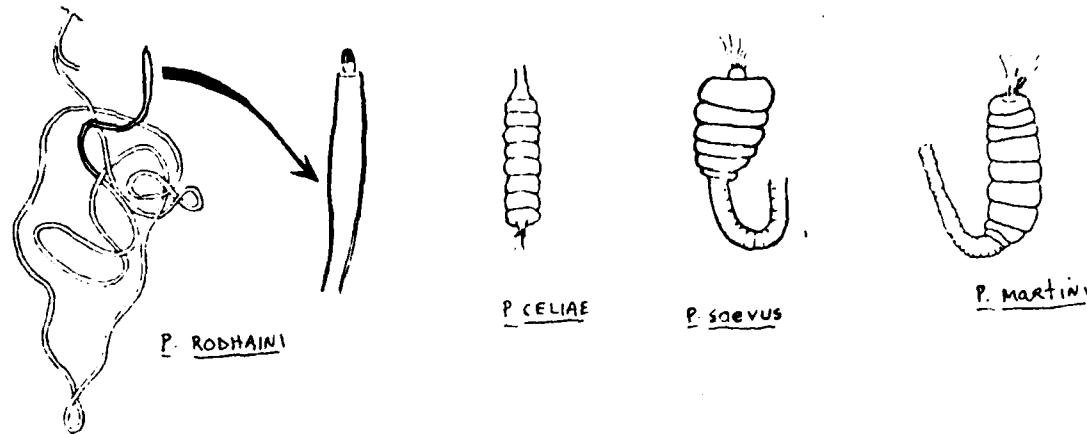


Wing length 1.9-2.5. Antennal segment 3, 0.25-0.30 mm P. langeroni orientalis

(= orientalis of various authors)

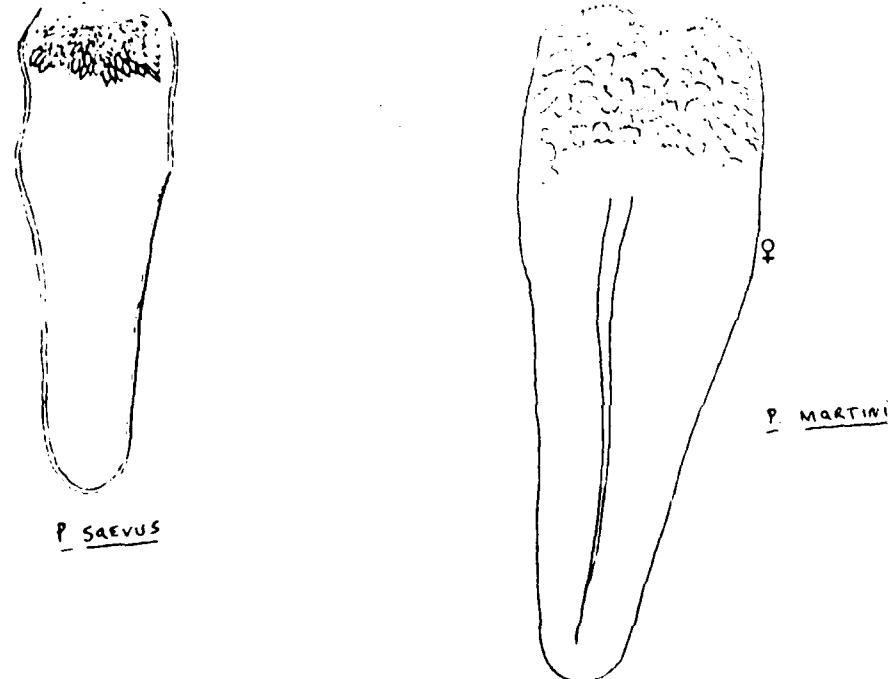
* FEMALE HAVE BEEN REPORTED BITING MAN

4. Spermathecae smooth-walled, nonsegmented (Subgenus Anaphlebotomus) P. rodhaini
Spermathecae with well defined segments 5

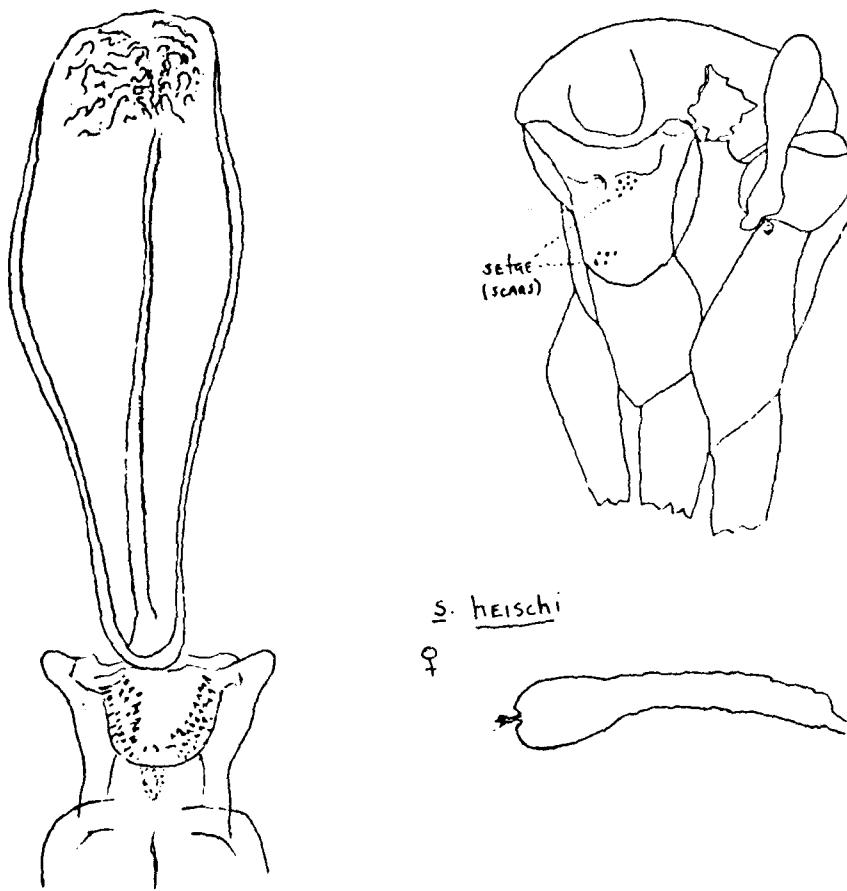


5. Spermathecae with 4-6 segments. Pharynx with armature of coarse teeth. (Subgenus Paraphlebotomus) P. saevus

- Spermathecae with 9-10 segments. Pharynx with ridges and small teeth. (Subgenus Synphlebotomus) P. martini
P. celiae
P. vansomerenae



6. Cibarium with lateral longitudinal and irregular rows of teeth. Pleural setae positioned as shown. (Subgenus Parvidens). S. heischii



Spermathecae otherwise, with no setae; pleural setae absent*. . . . 9



GRASSOMYIA - TYPE
SPERMATHECA

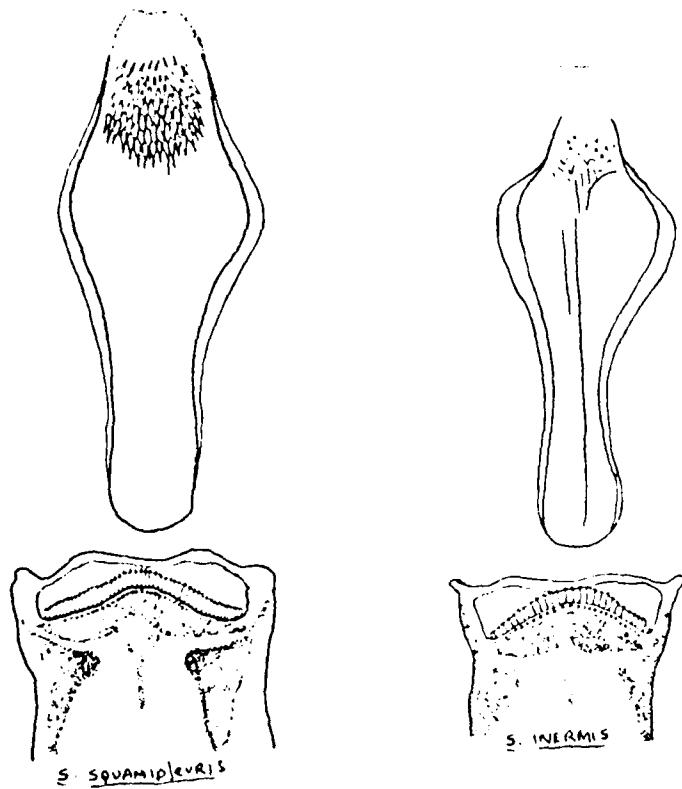
S SQUAMIPLEURIS

* SERGENTOMYIA AFRICANA magma
(couplet 20) MAY ALSO HAVE ONE
GROUP OF PLEURAL SETAE BUT
THE SPERMATHECAE ARE QUITE
DIFFERENT.

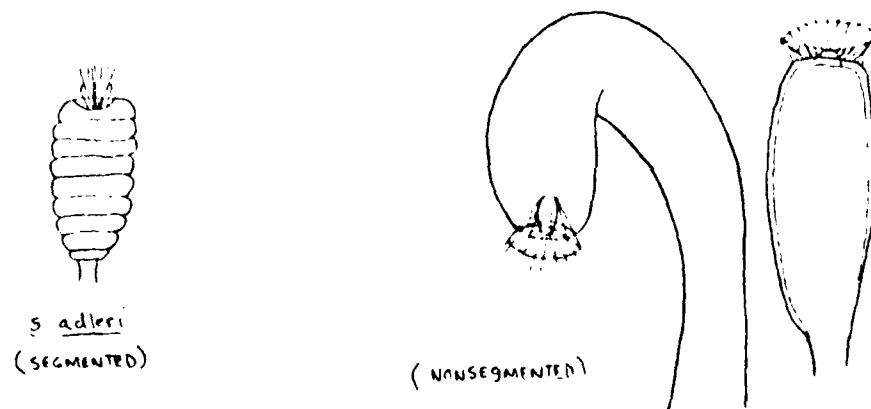
* RARE MAN-WITER

8. Pharynx with numerous stout teeth. Pleural setae on mesanepisterum (SEE ~~KEY~~ KEY).
17 to 35. Cibarium with 20 to 26 relatively long teeth
P. squamipleuris

Pharynx with only a few minute teeth. Pleural setae on mesanepisterum (SEE ~~KEY~~ KEY)
1-9. Cibarium usually with fewer than 20 horizontal teeth
P. inermis

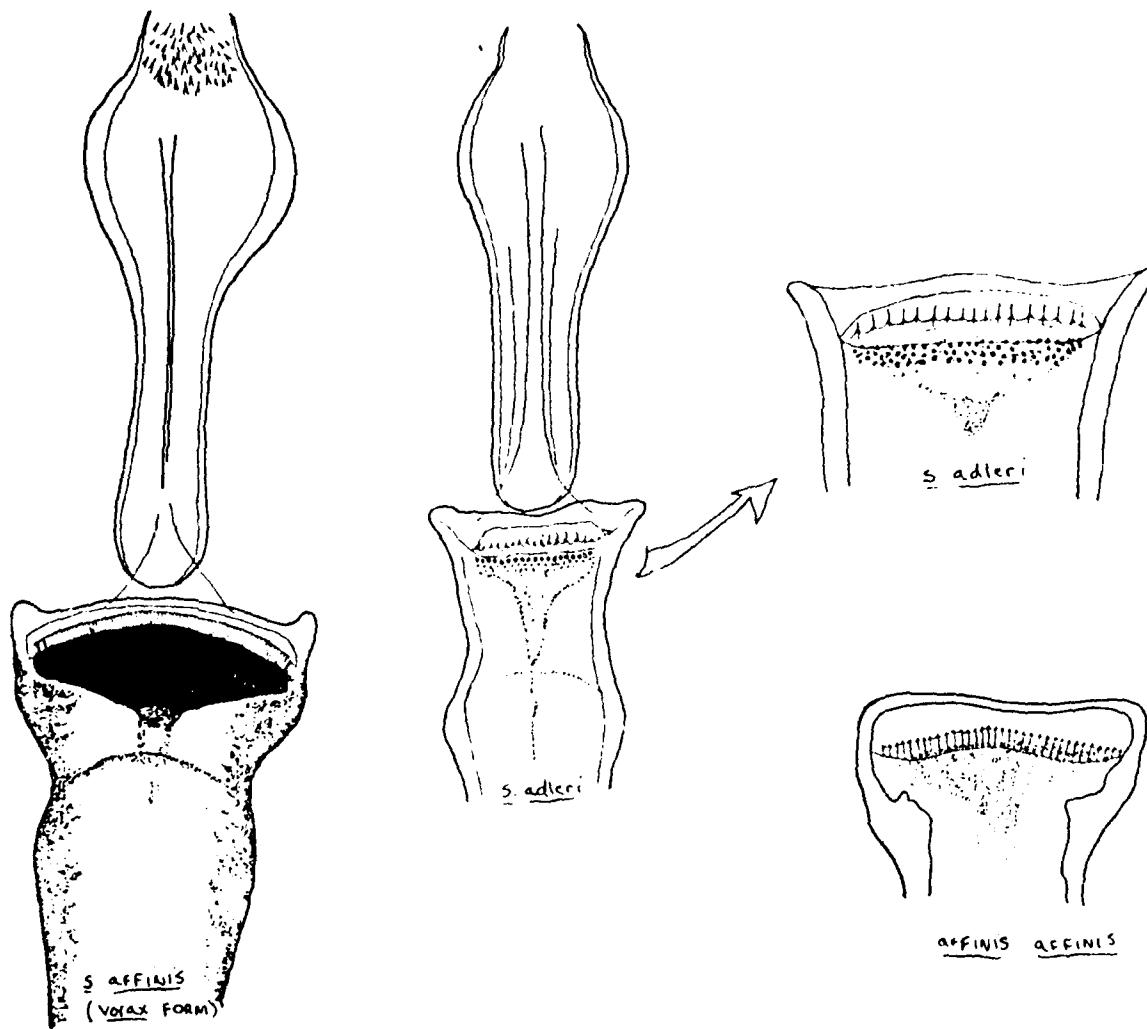


9. Spermathecae clearly segmented 10
Spermathecae smooth walled, nonsegmented 15



10. Front and hind femora with a row of conspicuous dark spines (SEE σ KEY).
Pharynx with about 50 teeth with long points; cibarium with
34-40 teeth in a comb-like row S. affinis*

Front and hind femora unarmed 11

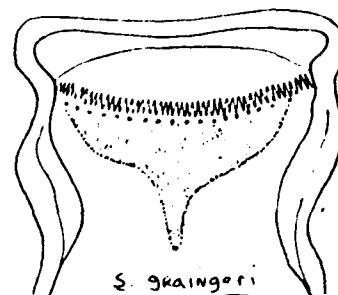
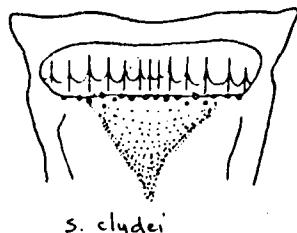
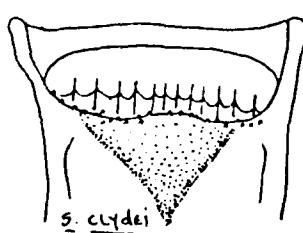


11. Cibarium with 3 or more rows of vertical teeth. . . . P. adleri

Cibarium with 0 to 2 rows of vertical teeth 12

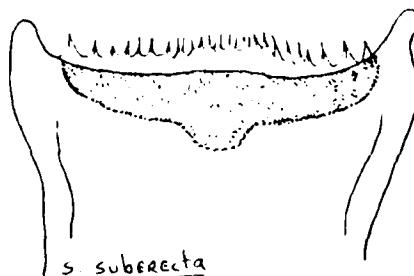
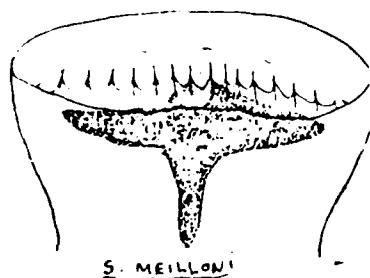
* S. affinis vorax ALSO OCCURS IN KENYA THE FEMALES OF THIS FORM HAVE 60 OR MORE HORIZONTAL TEETH IN THE CIBARIUM RATHER THAN 34 TO 40 FOR S. affinis affinis.

12. Cibarium with well developed row of teeth; no vertical teeth 13
Cibarium with vertical teeth 14



13. Cibarium with 14-16 stout, pointed teeth; pigment patch large and mushroom shaped *S. meilloni*

Cibarium with 25-27 teeth, the lateral ones larger and farther apart than the median teeth *S. suberecta*

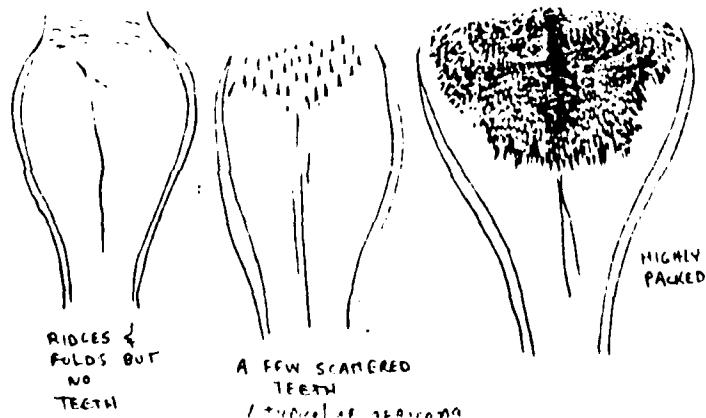


14. Cibarium with 25 or more horizontal teeth. Pigment patch in the form of a pointed helmet *S. graingeri*

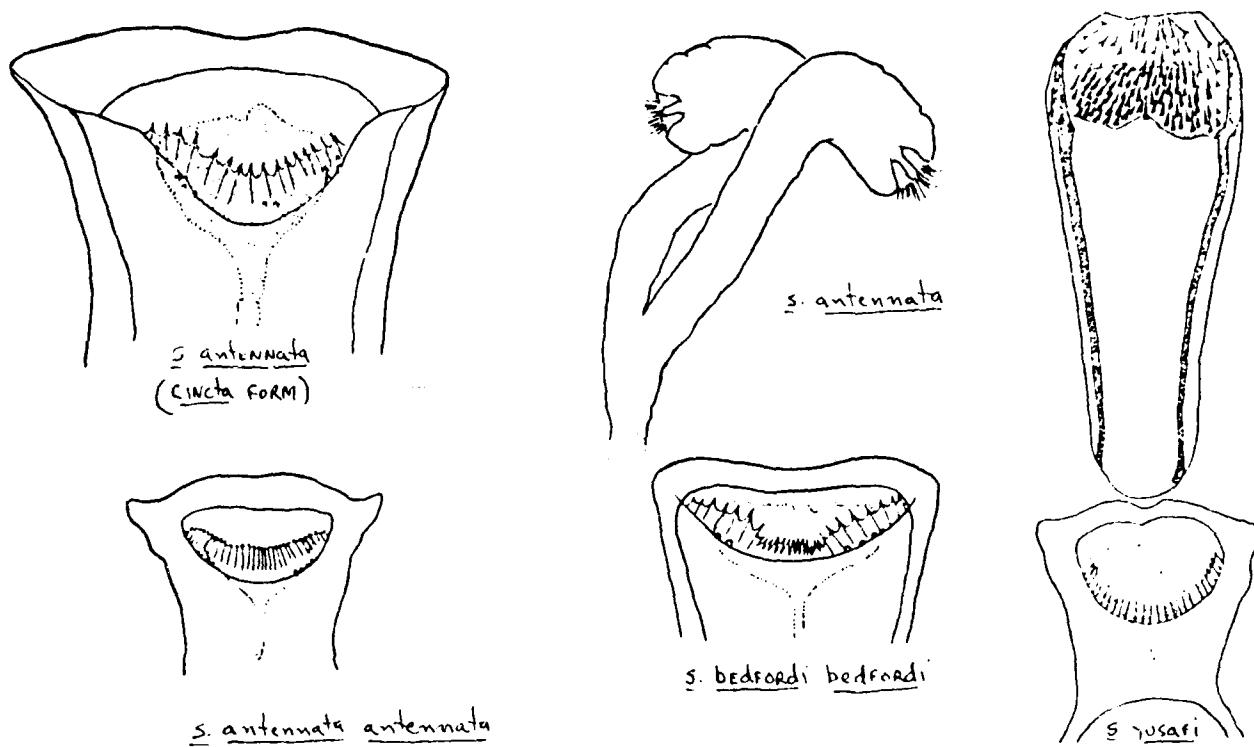
Cibarium with 20 or fewer horizontal teeth. *S. clydei*

15. Pharynx armed with numerous teeth in highly packed rows. 16

Pharynx unarmed or with only a few scattered distinct teeth 19

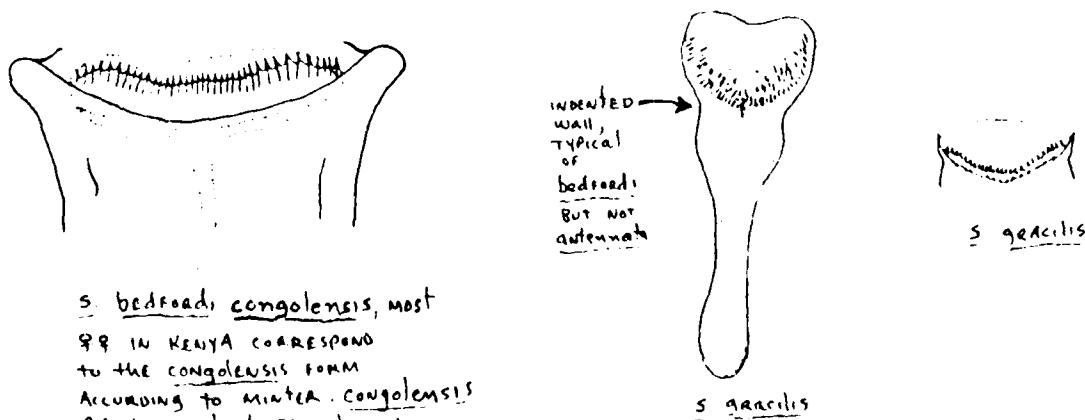


16. Cibarial teeth suboval, monomorphic 17
 Cibarial teeth in middle smaller than lateral teeth 18
 (N.b. S. antennata and bedfordi common; S. yusafi and gracilis rare)
17. Antennal segment III < .100 mm; palpal segment 4 somewhat >
 or = 3 S. antennata*
 Antennal segment III > .100 mm (coastal sp.) . S. yusafi (rare)

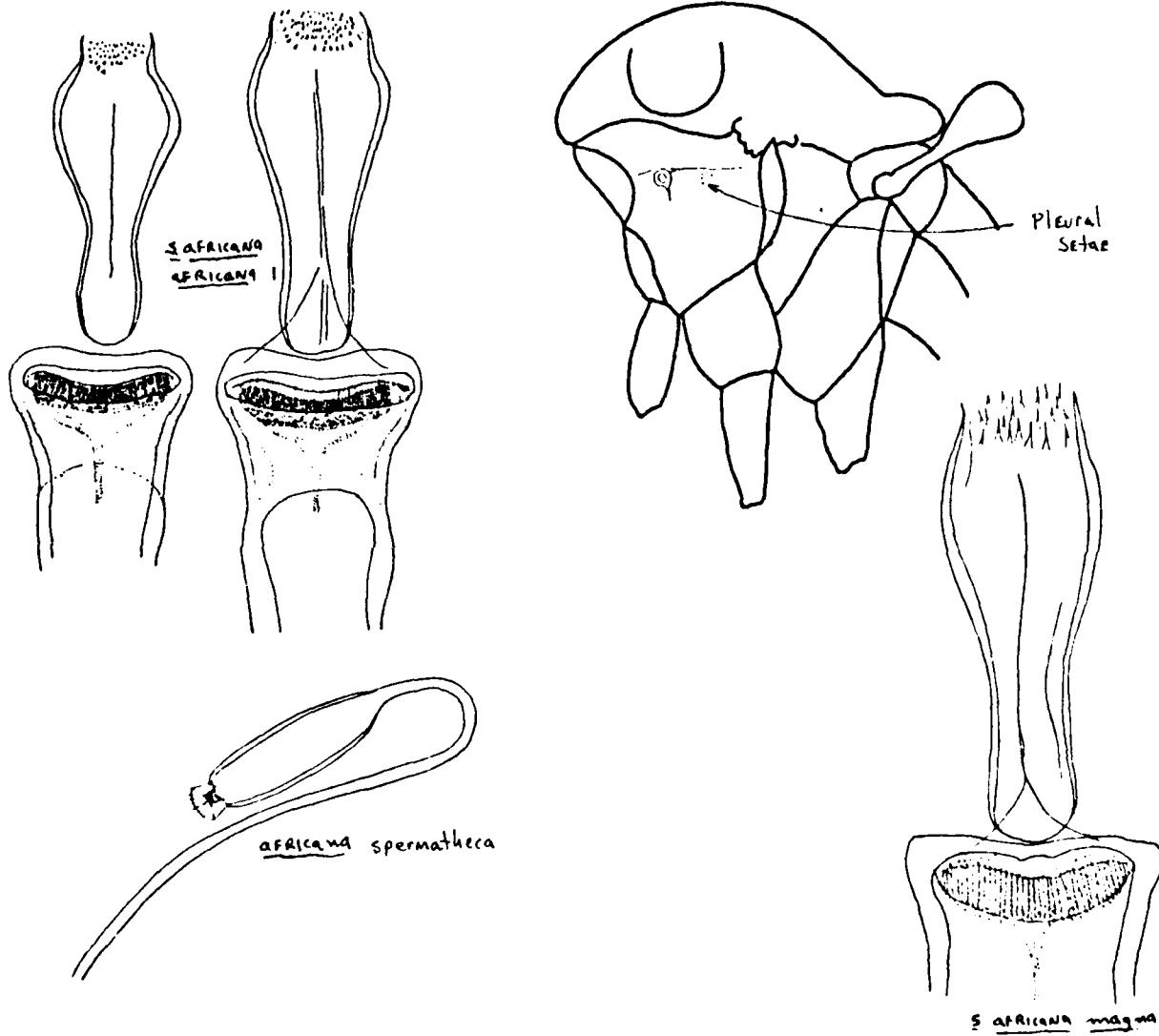


18. Cibarium with 26-28 small pointed teeth, the median ones
 smaller than laterals S. gracilis (rare)
 Cibarium with 24-28 teeth on an arc, the 12-13 median teeth
 small, the lateral teeth stout, palpal segment 4 clearly > 3
 S. bedfordi*

* S. bedfordi w/ indented pharyngeal wall (compare Quate, Fig. 19 B & 20C)

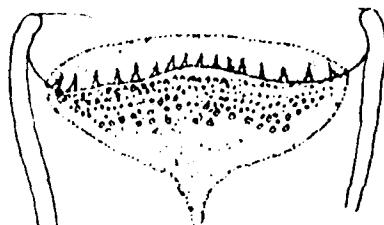
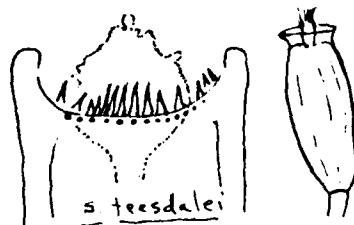


19. Pharynx armed with a small number of scattered denticles 20
Pharynx unarmed or with fine folds, ridges, or broad scales
having finely denticulate (dot-like) posterior borders 21
20. Gbarium armed with about 60 teeth; pigment plate black, often
obscuring teeth S. africana africana
Gbarium armed with not more than 45 teeth. Pigment patch usually
brown; 1-6 insertions (ie. setal scars) on thoracic pleurae
..... S. africana magna*



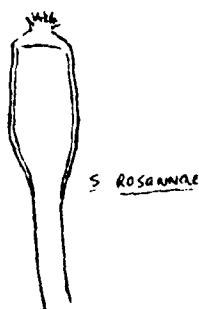
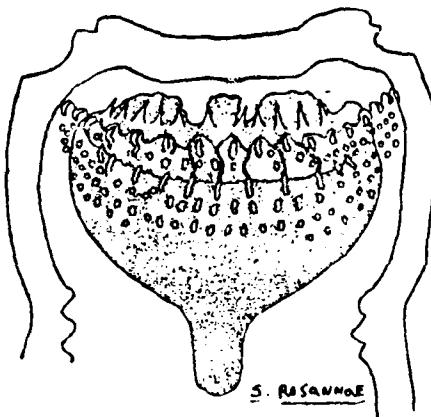
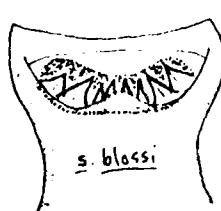
* Abonnenc (1972) elevates this subspecies to species rank, calling it S. magna. Not all specimens have pleural setae in Ethiopia. Specimens in Kenya may or may not show this variation. It is not clear whether one or both of the forms in couplet 20 occurs in Kenya.

21. Antennal segment III very long (.390-.44 mm) 22
Antennal segment III long (.09-.38 mm) 23
22. Cibarium with 11-12 teeth and only 1 row of anterior vertical teeth S. teesdalei
Cibarium armed with 16 strong teeth and w/ 6-8 rows of anterior vertical teeth. S. multidens



S. multidens

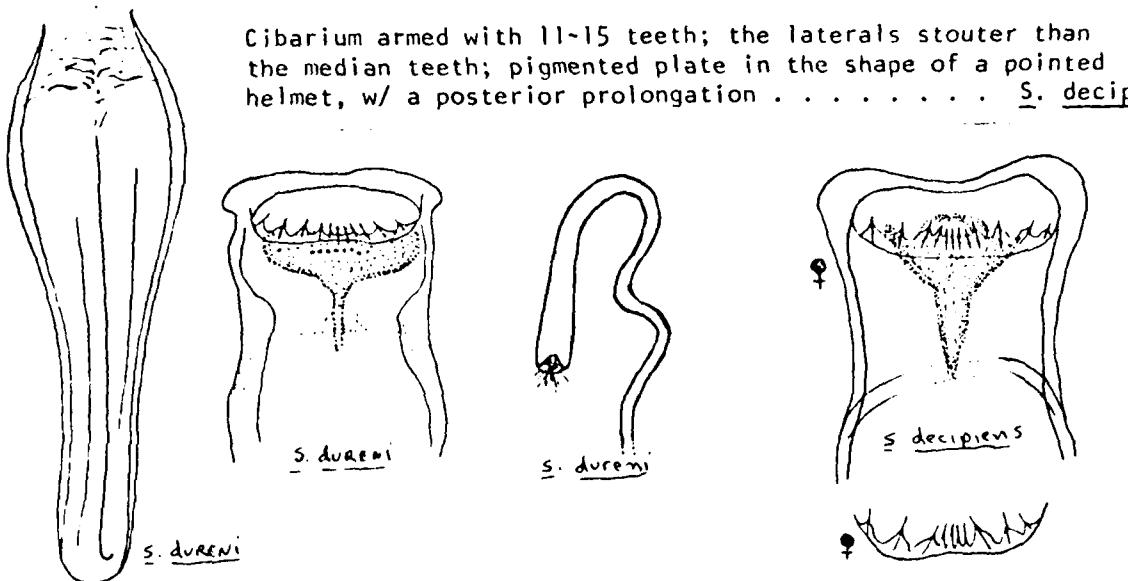
23. Pigmented plate well developed, filling the entire width of the cibarium 24
Pigmented plate narrow or absent 29
24. Cibarium with 8 to 18 teeth 25
Cibarium with 20 teeth. 28
25. 8 cibarial teeth, the 4 median ones short and narrow, the lateral teeth stout and wide pigmented plate bilobed. . S. blossei
10-15 cibarial teeth; pigment plate not bilobed 26



26. Cibarium armed w/ 10-12 short retractile teeth, scarcely visible; very stout, rounded tooth-like folds present anterior to the cibarial teeth; 3-6 rows of anterior vertical teeth. Pigmented plate large and massive S. rosannae
No rounded tooth-like folds in the cibarium 27

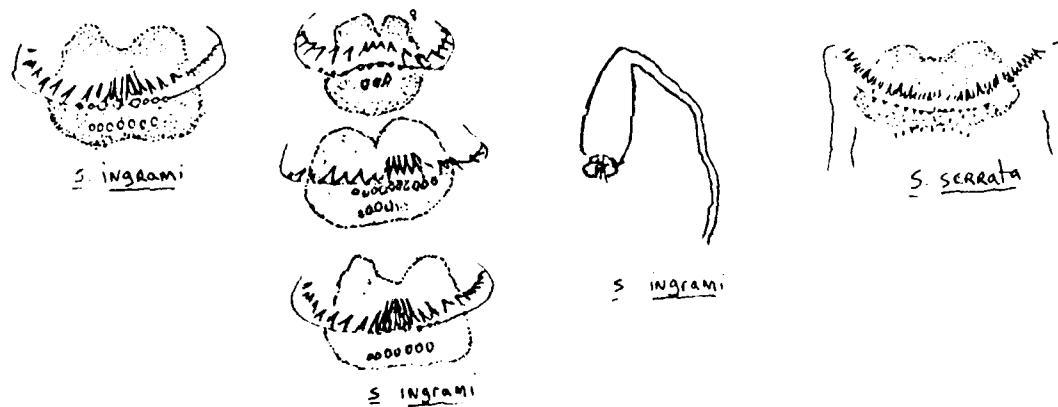
27. Cibarium armed w/ 12-14 broad, needle-like teeth and 1 or 2 rows of anterior vertical teeth; pigmented plate a narrow transverse band S. dureni

Cibarium armed with 11-15 teeth; the laterals stouter than the median teeth; pigmented plate in the shape of a pointed helmet, w/ a posterior prolongation S. decipiens



28. Cibarium armed with 24-28 polymorphic teeth, 2 or 3 rows of anterior vertical teeth, of which the central teeth are noticeably larger; a few erect hairs on abd. tergites 2-6 S. ingrami

Cibarium with 40-52 teeth and 3 rows of subequal vertical teeth. No erect hairs on abdominal tergites 2-6 . . . S. serrata

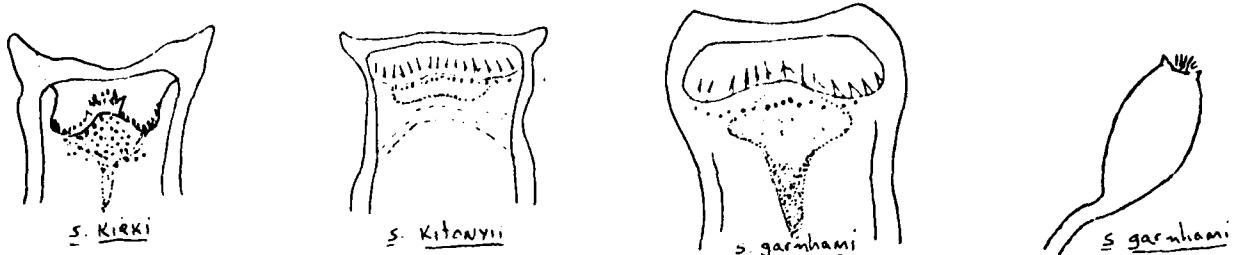


29. Cibarium with 1 or several rows of vertical teeth, or an unorganized group of teeth 30

Cibarium without vertical teeth 32

30. Cibarium with 22-24 polymorphic teeth on an M-shaped sinuous line; numerous vertical teeth not arranged in rows S. kirki

Cibarium with a transverse row of vertical teeth. 31

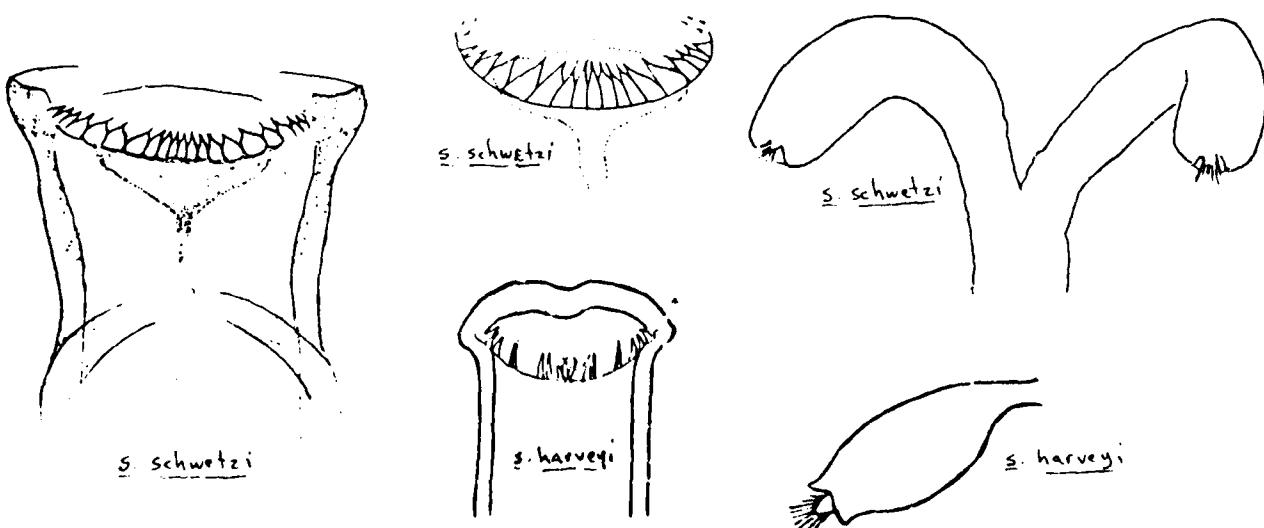


31. Pigment patch heart-shaped; antennal segment 3 0.26 - 0.32 mm long; cibarium with 15 long subequal needle-like teeth on a slightly sigmoidal. A row of anterior vertical teeth. S. kitonyii

Pigment patch, top-shaped; cibarium 13 stout pointed teeth on a slightly sinuous line and a row of 14-16 vertical teeth S. garnhami (A RARE MAN-biter)

32. Spermatheca tubular with the terminal knob embedded in the body. Cibarium with 16-18 teeth. S. schwetzi*

Spermatheca cylindrical. Cibarium with 11 teeth in an arc S. harveyi



* SOMETIMES bites MAN. THE ATYPICAL AND TYPICAL FORMS ARE SEPARATED ONLY IN THE

Appendix II

Sand Flies of the Central Amazon of Brazil. 2. Description of Lutzomyia (Trichophoromyia) ruii n. sp. (Diptera: Psychodidae)¹

Jorge R. Arias²

David G. Young³

Abstract

Lutzomyia (Trichophoromyia) ruii Arias & Young n. sp. is described and illustrated from both sexes commonly found near Manaus, Brazil. Females are not anthropophilic. Information on seasonal distribution is given. Lutzomyia (Trichophoromyia) melloi (Causey & Damasceno) is a junior synonym of Lutzomyia (T.) inini (Floch & Abonnenc) (NEW SYNONYM).

Introduction

Sherlock and Guitton (1970) reviewed the phlebotomine subgenus Trichophoromyia Barretto, 1962, described a new species, and provided a key to the males of 18 species. Two of them, Lutzomyia (T.) melloi (Causey & Damasceno, 1945) and Lutzomyia (T.) inini (Floch & Abonnenc, 1943) were separated in couplet 4 by genitalic characters. We now believe, however, that L. inini is a senior synonym of L. melloi based on recent determinations of males from Brazil and French Guiana by us and M. Emile Abonnenc (NEW SYNONYM). Recently, Young (1979) described 3 new Trichophoromyia spp. from southern Colombia. Here, we describe another species which is rather common in the vicinity of Manaus, Brazil.

Lutzomyia ruii is named in honor of Mr. Rui Alves de Freitas of the Instituto Nacional de Pesquisas da Amazônia for his dedicated service in the continuing study of phlebotomines and leishmaniasis in the Amazon Basin of Brazil.

Terminology follows that of Young (1979). The description is based on the holotype and allotype with ranges of some measurements of paratypes (111♂♂, 11♀♀) given in parentheses. Measurements are in millimeters.

Lutzomyia (Trichophoromyia) ruii Arias & Young, n. sp.

Fig. 1-9

Male: A large dark sand fly; pleura moderately pigmented, mesonotum strongly infuscated. Wing length 2.10 (2.06-2.18); width 0.56 (0.53-0.56). Cibarium with about 12, scattered dot-like remnants of vertical teeth; cibarial arch distinct only at sides, without pigment patch. Pharynx 0.18, unarmed. Head height ; width . Eyes large, separated by 0.126 or by distance equal to 7.1 facet diameters. Flagellomere I, 0.23 (0.21-0.24), combined length of II + III slightly longer than I; ascoids with very short distinct posterior spurs, the distal tips of ascoids on II reaching beyond flagellomere, on all flagellomeres except last 3. Labrum 0.23 (0.21-0.24) long. Length of palpal segments (holotype): 1 (0.04), 2 (0.10), 3 (0.40), 4 (0.06), 5 (0.14); palpal sensilla at end of segment 2 and along middle third of 3. Pleura with 15 (7-15) upper and 2 (2-4) lower episternal setae. Length of wing vein sections: alpha 0.56 (0.49-0.58), beta 0.28 (0.26-0.33), delta 0.42 (0.28-0.42), gamma 0.22 (0.22-0.28). Length of femora, tibiae and basitarsi of holotype: foreleg, 0.86, 1.13, 0.68; midleg, 0.79, 1.35, 0.83; hind leg, 0.86, 1.58, 0.90. Genitalia: style 0.23 long, with 4 major spines arranged as shown, no sub-terminal seta. Coxite ca. 0.39 long x 0.12 wide, bearing a median group of ca. 45 long slender hairs. Paramere simple as shown. Aedeagus broad, well pigmented. Genital pump 0.18 (0.15-0.18)

long, each filament 0.87 (0.87-1.02) long or 4.8 x length of pump, tip simple. Lateral lobe 0.38 (0.38-0.40) long. Cercus as shown.

Female: Wing length 2.20 (2.20-2.40); width 0.64 (0.64-0.75). Cibarium, with 10-12 slender pointed, more or less equidistant horizontal teeth and 20-30 vertical teeth, median teeth larger than others; cibarial arch complete; pigment patch well infuscated. Pharynx 0.19 (0.18-0.20) long, unarmed. Head height ; width . Eyes large, separated by 0.55 or by distance equal to 7.2 facet diameters. Flagellomere I 0.20 (0.20-0.25) long, II + III slightly longer than I; ascoids as in male, on all flagellomeres except last 3. Labrum length 0.34 (0.34-0.43). Length of palpal segments of allotype: 1 (0.05), 2 (0.15), 3 (0.19), 4 (0.06), 5 (0.14); palpal sensilla as for male. Pleura with 9 (9-17) upper and 2 (2-4) lower episternal setae. Length of wing vein sections: alpha 0.70 (0.64-0.78), beta 0.27 (0.27-0.30), delta 0.49 (0.41-0.55), gamma 0.25 (0.20-0.29). Length of femora, tibiae and basitarsi of allotype: foreleg, 0.75, 1.01, 0.71; midleg, 0.75, 1.24, 0.83; hindleg, 0.83, 1.46, 0.94. Spermathecae annulated except for smooth subterminal portion; individual ducts very long, ca. 6.5 x length of spermatheca; common duct shorter than spermatheca.

Type Data: Holotype male (DCDC-419, no. 1).

Reserva Ducke, 26 km E of Manaus, Amazonas, Brazil; light trap; 6 Dec. 1977; J. Arias, R. Freitas & J. Vidal colls. Allotype female (Ducke-CDC, no. 2). Same data but taken on 18 June 1976.

Paratypes (120♂♂, 30♀♀). Reserva Ducke, Reserva Campinas (43 km NE of Manaus), at km 30 and 4 km S of km 56 Rodovia Torquato Tapajós-

Estrada AM-010; CDC light traps, flight traps, soil emergence traps, and animal burrows; 1974 to 1979, J. Arias et al. colls.

Holotype, allotype and 20 paratypes to be deposited at Instituto Nacional de Pesquisas da Amazonia, Manaus. Other paratypes in Brazilian collections at Universidad Federal de Minas Gerais and São Paulo; Instituto Oswaldo Cruz, Rio de Janeiro; Instituto Evandro Chagas, Belém; Museu Paraense Emilio Goeldi, Belém. Remaining paratypes in collections of U.S. National Museum (Natural History), Washington; Florida State Collection of Arthropods, Gainesville, Florida; Instituto de Salude Publica, Lima; British Museum (Natural History), London. All specimens slide mounted.

Discussion:

We associated the sexes of L. ruii on the basis of collecting data. No other Trichophoromyia spp. were taken at the study site 30 km NE of Manaus during a 62 week period of time.

This species occurs with Lutzomyia (Trichophoromyia) ubiquitalis at Reserva Campinas. The males differ readily by the shape of the parameres (more slender in ubiquitalis) and by the length of the genital filaments (shorter than 4X length of pump for ubiquitalis; over 4.5X for ruii).

The females of ruii and ubiquitalis closely resemble each other in nonsexual traits of color, cibarial armature and wing venation (This generally is true for the other Trichophoromyia females as well). However, the shorter sperm ducts and larger terminal knob of the spermathecae of ubiquitalis (Fig. 10) easily serve to distinguish the females. Floch and Abonnenc (1943, 1952)

describe and illustrate the ubiquitalis female as Phlebotomus cauchensis, an accepted junior synonym.

From the Trichophoromyia males having a median tuft of numerous coxite setae such as L. inini, L. auraensis (Mangabeira, 1942) and L. loretonensis (Llanos, 1964), L. ruii differs in the shape of the parameres (see Sherlock & Guitton, 1970, for figures of these other males).

Light trap catches from the site, 30 km NE of Manaus, during a 62 week period (Fig. 11) indicated population peaks of L. ruii adults from August-November, 1977 and February-March and August, 1978. Females do not attack man and their preferred hosts remain unknown.

We wish to thank M. Emile Abonnenc for identifying a male of L. inini collected in French Guiana by Dr. T.H.G. Aitken. Also, we appreciate the valuable assistance of Mr. João Ferreira Vidal in the field and laboratory.

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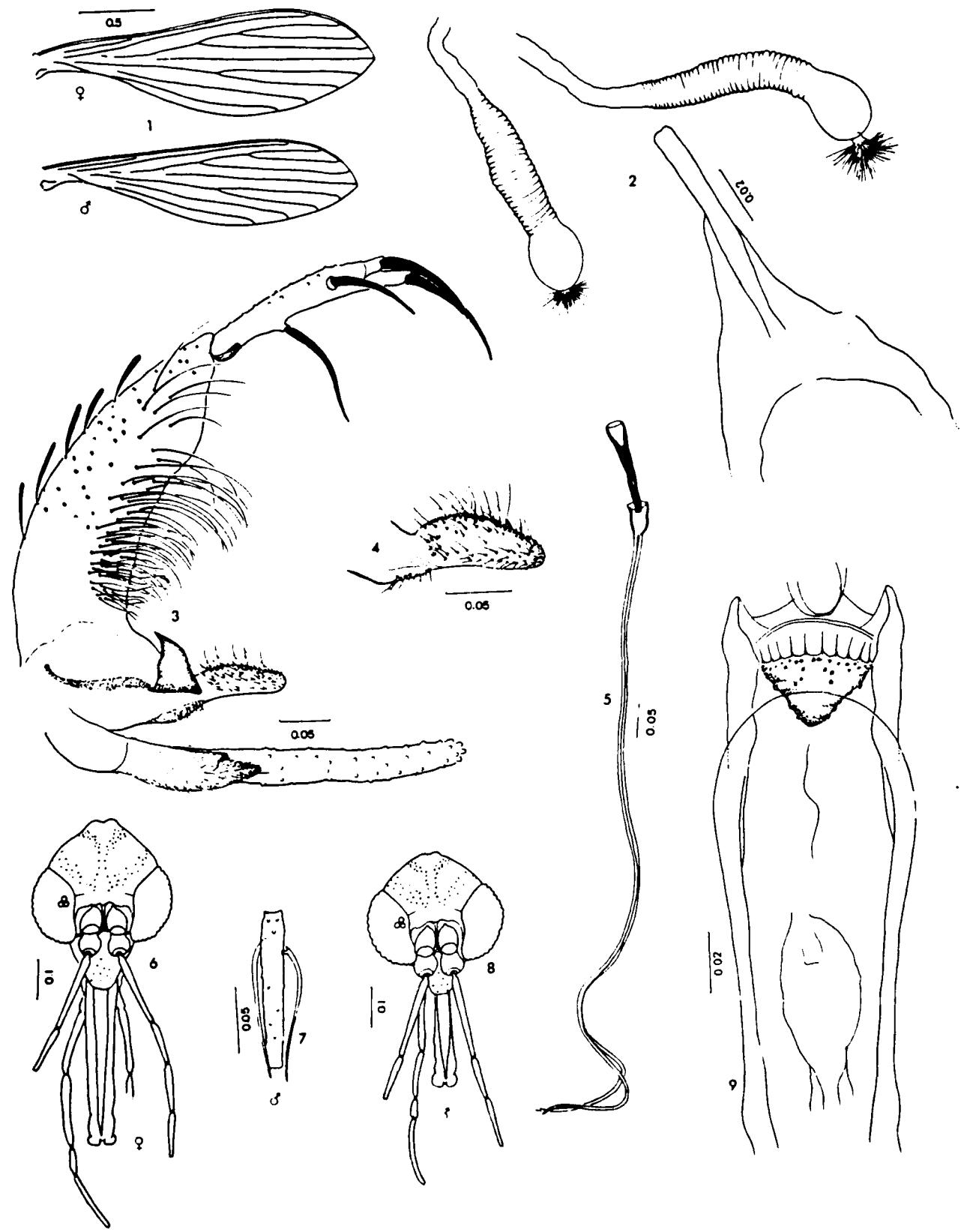
Young, D.G. 1979. A review of the bloodsucking psychodid flies of Colombia (Diptera: Phlebotominae and Sycoracinae). Univ. of Florida Agr. Exp. Sta. Tech. Bull. 806. 266p.

Footnotes

- 1) Research was partly funded by CNPq Grant SIP 08/131; INPA Project 2017/103, and U.S. Army Research and Development Contract DADA 17-72-C-2139.
2. C.P. 478, Manaus, 69000, Amazonas, Brasil (Address for reprint requests).
3. Dept. of Entomology and Nematology, University of Florida, Gainesville, Florida U.S.A. 32611.

Figures

- Fig. 1-9. Lutzomyia ruii Arias & Young n. sp. 1) Female and male wing.
2) Spermathecae and genital fork, sperm ducts not visible.
3) Male genitalia, lateral view. 4) Paramere, a different,
larger view than shown in Fig. 3. 5) Genital pump and fila-
ments. 6) Female head. 7) Male flagellomere II showing ascoids.
8) Male head. 9) Female cibarium. All figures drawn from
specimens found at Reserva Ducke. Scale in mm.
- Fig. 10. Spermatheca of Lutzomyia ubiquitalis from Belém, Pará, Brazil.
The other spermatheca was not drawn. Scale in mm.
- Fig. 11. Seasonal distribution of Lutzomyia ruii n. sp. based on light
trap catches at 1 m and 15 m heights above ground at Reserva
Ducke (1977-1978).



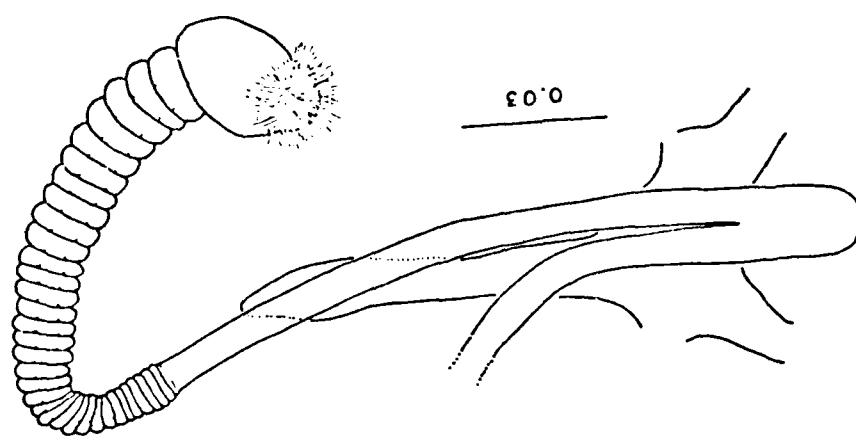


Fig. 10

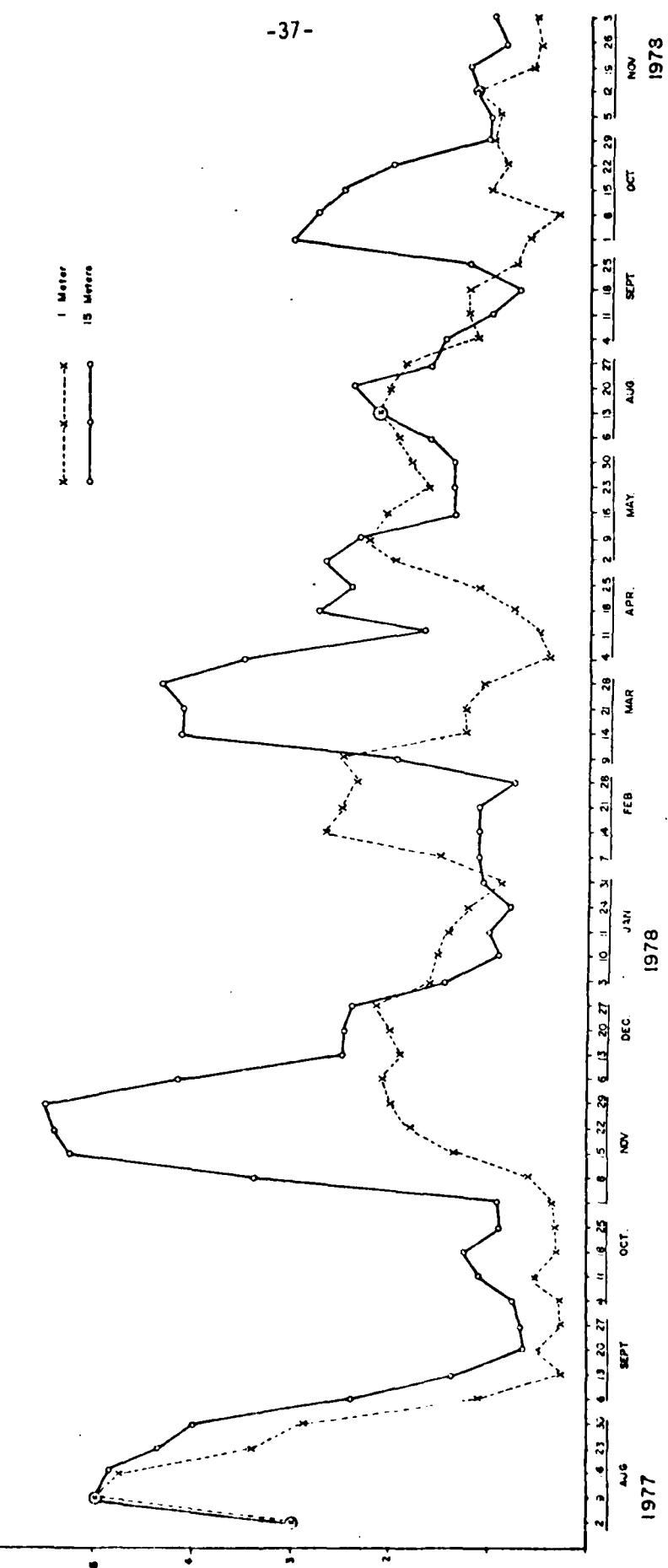


Fig.

Appendix III

A NEW PHLEBOTOMINE SAND FLY IN THE *Lutzomyia flaviscutellata* COMPLEX FROM NORTHERN BRAZIL (DIPTERA: PSYCHODIDAE)¹

D.G. Young²

J.R. Arias³

Sand flies of the *Lutzomyia (Nyssomyia) flaviscutellata* complex are important as vectors of *Leishmania mexicana* to small mammals and man in the neotropics. Lainson and Shaw (1968) and Ward et al. (1973, 1977) incriminated *Lu. flaviscutellata* (Mangabeira) as the vector of *Leishmania mexicana amazonensis* in Brazil. Tikasingh (1975) recovered this parasite, or a similar one, from wild caught *flaviscutellata* females in Trinidad. *Lu. olmeca clmeca* (Vargas and Nájera) in Mexico and Central America and *Lu. o. bicolor* Fairchild and Theodor in Panama are proven or suspected vectors of *Leishmania mexicana mexicana* and *L. m. aristedesi*, respectively. Lainson and Shaw (1979) reviewed the epidemiology of these diseases, noting that "it appears that subspecies of *L. mexicana* are principally parasites of sylvatic rodents . . . transmission occurs at ground level, where man also becomes infected."

Aitken et al. (1975) isolated Pacui virus and several other arboviruses from wild caught *Lu. flaviscutellata* females near Belém, Brazil. The Disney trap, an animal-baited oil trap routinely used by them and other workers, is excellent for capturing sand flies in this complex (Disney, 1966).

Fairchild and Theodor (1971) and Lewis (1975) provided information on the taxonomy and distribution of these forest sand flies, the latter author observing among other things, "the presence of many erect hairs

on abdominal tergite 2, while there are none on segment 5. These features and those already known make this complex a remarkably distinct one." The striking coloration of these sand flies, i.e. the pale pleura, coxae and posterior mesonotum contrasting with very dark head, abdomen, and legs, often allows workers to identify specimens without the aid of magnification.

The form described here as a subspecies of *Lu. olmeca* occurs with *Lu. flavigutellata* at Manaus, Brazil, and vicinity. All measurements are given in millimeters.

Lutzomyia olmeca nociva Young and Arias n.ssp.

Fig. 1-10

Male holotype: Wing length 1.89; width 0.55. Except for pale coxae, pleura, posterior part of mesonotum, rest of insect dark. Head height from vertex to tip of clypeus, 0.36; width 0.33. Eyes large, separated by only 0.04 or by distance = to 2 facet diameters. Interocular suture absent. Flagellomere I, 0.31 long, combined length of II + III = 0.29; ascioids simple, tips of those on II ending before end of flagellomere, on all flagellomeres except last 3. Labrum 0.16 long. Length of palpal segments: 1 (0.027), 2 (0.081), 3 (0.119), 4 (0.054), 5 (0.086); palpal sensilla apparently restricted to middle third of palp 3. Cibarium with about 20 dot-like remnants of teeth, mostly at sides when viewed as in Fig. 10, no cibarial arch or pigment patch. Pharynx 0.19 long, unarmed. Pleura with 7-8 upper and 2 lower episternal setae. Length of wing vein sections: alpha (0.49), beta (0.24), delta (0.10), gamma (0.11). Length of femora, tibiae and basitarsi:

foreleg, 0.78, 1.30, 0.76; midleg, 0.71, 1.40, 0.93; hindleg, 0.83, 1.50, 1.02. Abdominal tergite 2 with ca. 20 erect hair sockets, tergite 3 with fewer, none visible on other tergites.

Genitalia. Style 0.15 long, with 4 major spines, basal pair inserted slightly beyond middle of segment; no subterminal seta.

Coxite 0.29 long, without persistent hairs. Paramere simple.

Aedeagus subtriangular, well pigmented throughout, acute tip.

Genital pump 0.116 long, each filament 0.35 or ca. 3X length of pump. Lateral lobe 0.24 long. Cereus as shown.

Female allotype. Wing length 1.98; width 0.56. Coloration as for ♂.

Head height 0.47 (including 0.17 long clypeus); width 0.33. Eyes large, separated by 0.05 or by distance = to ca. 2.5 facet diameters.

Interocular suture reduced to short stub. Flagellomere I 0.28 long, combined length of II + III = 0.24; ascoids as in ♂ but absent from terminal 2 flagellomeres. Labrum 0.28 long. Ventral maxillary teeth 25; lateral teeth 13. Length of palpal segments:

1 (0.035), 2 (0.116), 3 (0.159), 4 (0.060), 5 (0.110); palpal sensilla at middle third of segment. Cibarium with 9 horizontal pointed teeth, a few lateral teeth and ca. 36 ventral teeth, median ones larger than others; cibarial arch complete; pigment patch well infuscated, shaped as shown. Pharynx 0.21 long, unarmed. Pleura with 4 upper and 1 lower episternal setae. Length of wing vein sections: *alpha* (0.52), *beta* (0.26), *delta* (0.06), *gamma* (0.09). Length of femora, tibiae and basitarsi: foreleg, 0.83, 1.24, 0.80; midleg, 0.73, 1.47, 0.90; hindleg, 0.83, 1.50, 0.98. Abdominal tergite 2 with 30+ erect hair sockets, tergite 3 with 28, few or no erect setae on other tergites. Spermathecae

with 9-12 segments increasing in size from base to apex, terminal knob oblong, its length at least twice width; individual ducts nearly as long as common duct. Stem of genital fork very broad, blade-like.

Type Data (All material from Amazonas State, Brazil). Holotype ♂.

26 km E of Manaus at Reserva Ducke, 19 March 1979, flight trap, D.G. Young. Allotype ♀. 243 km E of Manaus at Rio Urubu, 13 March 1979, flight trap, D.G. Young, J.R. Arias et al. Paratypes. 2♂♂, 30 km E of Manaus, 3 Oct. 1974, rat-baited Disney trap, J.R. Arias. 3♂♂, Mauá, Estrada do Aleixo (km 10), Manaus, 18 Sept. 1974, lizard-baited Disney trap, J.R. Arias. 3♂♂, 18♀♀, 243 km E of Manaus at Rio Urubu, 12-14 March 1979, light and flight traps, human bait, D.G. Young, J.R. Arias et al. 8♂♂, 12♀♀, 26 km E of Manaus at Reserva Ducke, 16-19 March 1979, flight and light traps, D.G. Young, J. Vidal and R. Alves de Freitas. 2♂♂, 3♀♀, 43 km NE of Manaus at Reserva Campinas, 23 March 1979, light and flight traps, D.G. Young, J. Vidal and R. Alves de Freitas. The sub-specific name, "nociva," meaning hurtful or injurious, refers to the bloodsucking habit of the females.

Discussion:

The *flaviscutellata* complex of the subgenus *Nyssomyia* Barretto now consists of 5 named and 1, or possibly 2, unnamed forms. These are: *Lu. flaviscutellata* (Mangabeira, 1942) from South America, *Lu. elmeae elmeae* (Vargas and Diaz-Najera, 1959) from Mexico and Central America, *Lu. o. bicolor* Fairchild and Theodor, 1971, from Costa Rica to northern South America, *Lu. o. nociva* n.ssp. from North Central Brazil, and *Lu. inornata* Martins, Falcão and da Silva, 1965, from Brazil. Specimens

from Salvador, Bahia, Brazil (Sherlock and Carneiro, 1962, not *flaviscutellata* Mang.) and from Itiatuba, Pará, Brazil (Lewis, 1975) require further study before their status can be determined.

Lu. inornata, known from only the male, is structurally similar to *flaviscutellata* but the scutellum is dark rather than pale according to Martins et al. (1965). This feature, if consistent, also serves to separate this species from the subspecies of *olmeca*. Through the kindness of M. Emile Abonnenc and Prof. Nicole Leger, we examined a male, tentatively identified and recorded as *inornata* from Oyapock, French Guiana (Leger et al., 1977, Fig. 2). We believe, however, that the specimen is conspecific with *flaviscutellata* because the scutellum is paler than the mesonotum, palp 5 is shorter than palp 3, and the rounded tips of the genital filaments are similar to those of some *flaviscutellata* males from Pará, Brazil. These males, unlike those of *olmeca* and subspecies, have large genital pumps which are almost as long as the lateral lobes (Table 1).

Palpal segment 5 of *Lu. o. bicolor* and *Lu. o. nociva* males is subequal in length to palp 2; whereas palp 5 of *Lu. o. olmeca* is nearly as long as palp 3. The male of *nociva* is smaller than the other subspecies based on the length of the wings, flagellomeres, and lateral lobes of the genitalia. Also the narrow interocular distance of *nociva* males is useful in separating this subspecies from the others (Table 1).

Features of the spermathecae, cibarial armatures, palpi, and antennae are diagnostic for the *flaviscutellata* complex females (Fairchild and Theodor, 1971). The broad terminal knobs of the spermathecae of *Lu. o. olmeca* and the *flaviscutellata* complex female of Sherlock and Carneiro (1962) differ from the oblong knobs of the other females,

especially *nociva* (Figs. 10, 11, 12). Females of *nociva* and the nominate subspecies are distinguished from the others in the complex in having the individual and common sperm ducts subequal in length. The individual sperm ducts of the other females are markedly shorter than the common duct. Additional measurements and meristic characters for each taxon are shown in Table 1.

It should be noted that the broad, blade-like stem of the genital fork reaches its greatest development in *nociva* females. Not all are as wide as that shown in Fig. 7, but this is apparently due to viewing the structure at an improper angle.

The status of *Lu. o. nociva* may change after more is learned about its geographic distribution, especially in relation to that of *Lu. o. bicolor* which occurs in the Amazon basin at Leticia, Colombia, and Napo Province, Ecuador (Young, 1979). At present, there is no evidence indicating that the *olmeca* subspecies have sympatric distributions. *Lu. flaviscutellata*, however, does occur with two of the *olmeca* subspecies in Amazonia, supporting its position as a valid species and raising interesting questions about resource partitioning and disease relationships.

Acknowledgments

Mr. Alves de Freitas and Mr. João Ferreira Vidal collected and prepared many of the specimens. We also thank Prof. N. Leger and M. Emile Abonnenc for sending us phlebotomines from French Guiana for study.

Taxon	<i>Lu. flaviscutellata</i>	<i>Lu. inornata</i>	<i>Lu. olmeca olmeca</i>	<i>Lu. olmeca bicolor</i>	<i>Lu. olmeca nociva</i>
Character	♂♂	♂	♂♂	♀♀	♂♂
Interocular distance	0.048- 0.055 $(\bar{x} = 0.51)$	0.050- 0.068 $(x = 0.62)$? $(\bar{x} = 0.105)$	0.098- 0.110 $(\bar{x} = 0.106)$	0.106- 0.113 $(\bar{x} = 0.109)$
n = 10	n = 10			n = 10	n = 10
Length of sagittal lobe II	0.29-0.32	0.30-0.33	0.28	0.53	0.37-0.42
Number of horizontal teeth in barium	--	6-7	--	--	10-12
Length of penital lobe	0.20	--	0.19	0.13-0.16	--
Length of ateral lobe	0.20	--	0.20	0.40	--

Table 1. Measurements and counts of some features of the *flaviscutellata* complex species (from Fairchild and Theodor, 1971; Martins et al., 1965 for *Lu. inornata*; this paper where sample size (n) is given). Measurements in millimeters.

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Footnotes

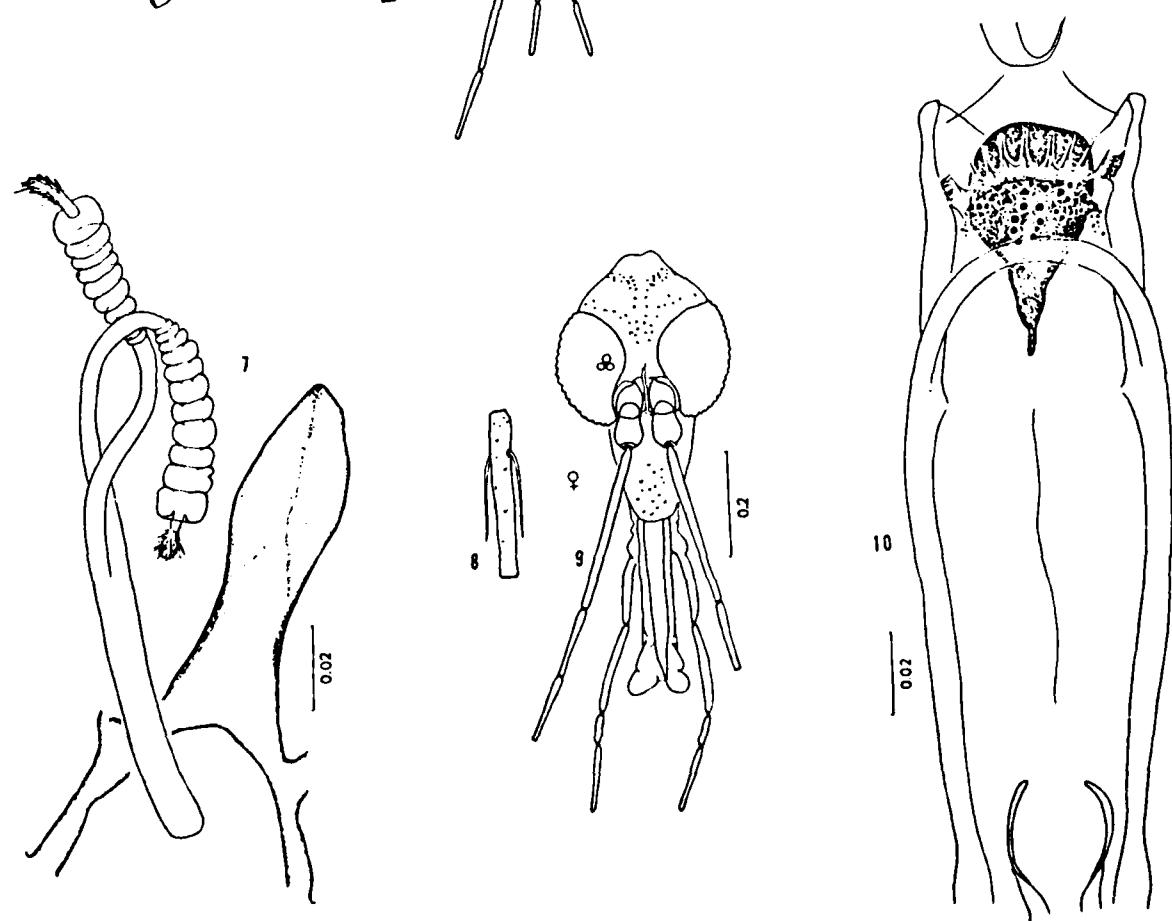
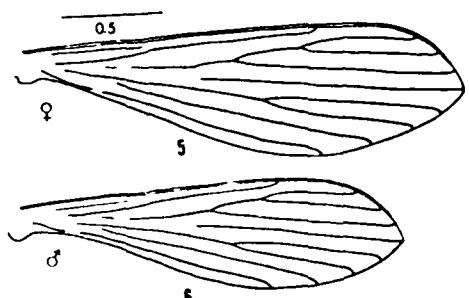
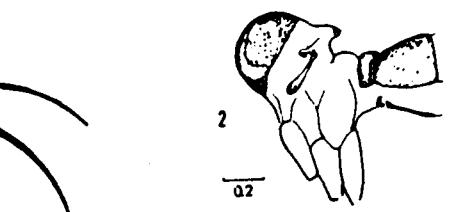
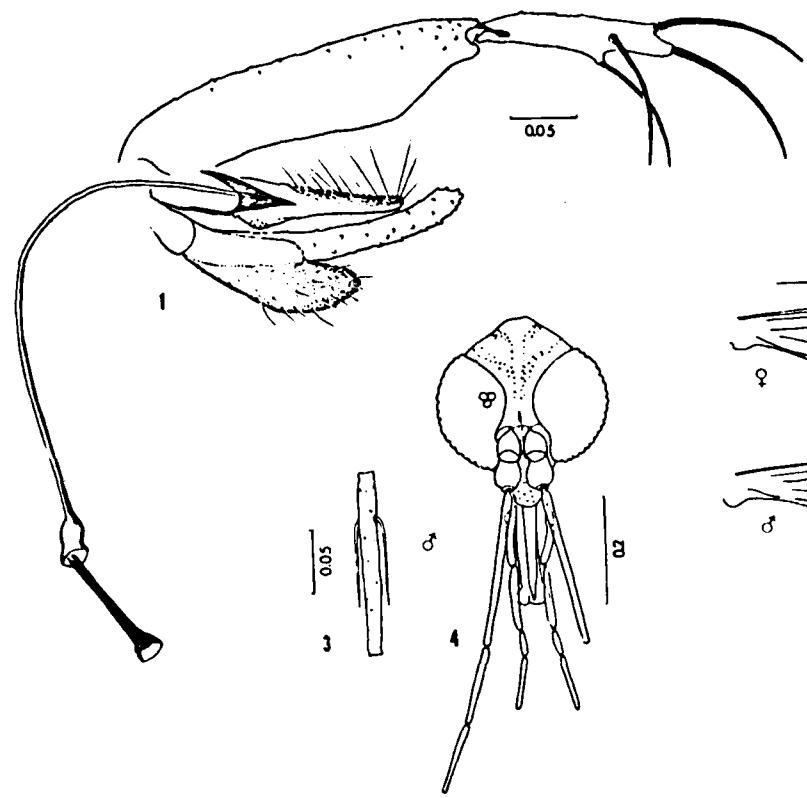
- 1) Research was partly funded by CNPq Grant SIP 08/131; INPA Project 2017/103, and U.S. Army Medical Research and Development Contract DADA 17-72-C-2139.
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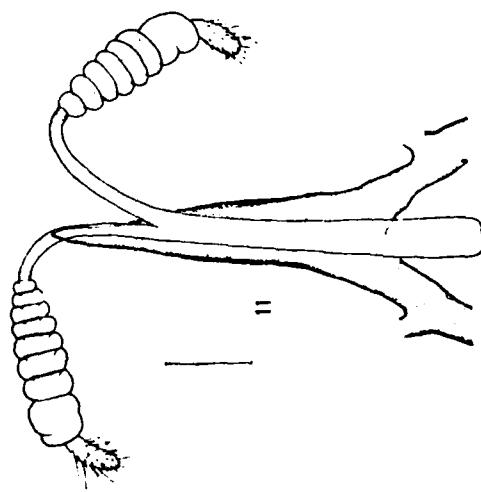
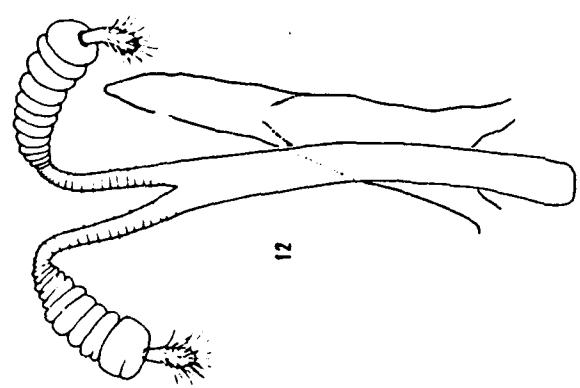
Figures

Figs. 1-10. *Lutzomyia olmeca nociva* n.ssp. 1) Holotype genitalia, lateral view. 2) Thorax and anterior abdomen of female. 3) Male flagellomere II. 4) Male head. 5) Female wing. 6) Male wing. 7) Spermathecae and genital fork. 8) Female flagellomere II. 9) Female head. 10) Female cibarium.
Scale in mm.

Fig. 11. Spermathecae of *Lutzomyia flavigutellata* of female collected with *Lu. olmeca nociva* at Rio Urubu, Brazil.

Fig. 12. Spermathecae of *Lutzomyia olmeca bicolor* (allotype).
Figures 11 and 12 drawn at same scale as Fig. 7.





Appendix IV

A Larval Diet for Rearing Phlebotomine Sand Flies (Diptera: Psychodidae)

Among the major problems in rearing phlebotomine sand flies is excessive larval mortality caused by fungal growth, improper diet or moisture, disease and/or other factors (Killick-Kendrick, R. 1978. *Acta Tropica* 35:297-313). None of the 600 or so species have been mass reared and only 6 or 7 have been reared in large numbers for more than 10 consecutive generations (Killick-Kendrick, op. cit.).

We have found that an aged 1:1 mixture (by wt. or vol.) of dry rabbit feces and Purina Rabbit Chow complete Diet #5315 (or Purina Horse Chow Checkers #3501) is an excellent diet for the larvae. The feces were obtained from laboratory rabbits fed on the Lab Rabbit Chow. Our attempts to rear four Lutzomyia spp. from the southern U.S.A. have been successful. We currently maintain closed colonies of Lutzomyia sp. near cruciata (Coq.), an autogenous strain (12 generations), L. anthophora (Addis) (6 generations) and L. shannoni (Dyar) (4 generations). We reared L. vexator (Coq.) in 1976 but purposely abandoned the colony after 4 generations.

The larval food is prepared by grinding the feces and whole chow into small pieces with a mortar and pestle. The final size of the particles ranges from 0.01 to 2.0 mm in diameter, the majority being about 0.5 to 1.0 mm. The dry mixture is evenly spread over the bottom of a petri dish or similar container to a depth of 0.5 to 1.0 cm, saturated

¹The chemical composition of these diets is available from the Ralston Purina Co., Checkerboard Square, St. Louis, Missouri 63188. Changes in composition during and after ageing due to microorganisms are not presently known.

with distilled water and placed in a glass desiccator at 22-30°C with 100% R.H. It is not sterilized. A crude inoculum of spores of the common bread mold, Rhizopus sp., is transferred from older diet to fresh using a wooden applicator. The desiccator is then covered with a tight fitting lid to prevent entry of phorid flies and other arthropods. The mixture is allowed to age for a month or more (Safyanova, V.M. 1964. Bull. Wld. Hlth. Org. 31:573-576). It is ready for use after most or all of the visible fungi disappears and when the odor becomes similar to that of rich humus. This ageing process eliminates the problem of excessive fungi which, by sheer density, can immobilize and kill larvae.

We rear 1 to 70 larvae in 7 dr (25 ml) polystrene snap-cap vials at temperatures ranging from 23-27°C and 80-100% R.H. The bottom half of each vial is filled with plaster of paris, allowed to dry, then saturated with distilled water. Gravid sand flies, usually 1 per vial, oviposit in these containers. The moist, aged medium is introduced into the vials anytime before egg hatch (about 1 g of medium for 50 eggs). As the larvae grow, we do not remove their feces but sometimes add more food or water. Little else is required for their care. Larval mortality, consistently less than 10%, results from factors other than improper nutrition such as accidents due to handling or excess water.

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